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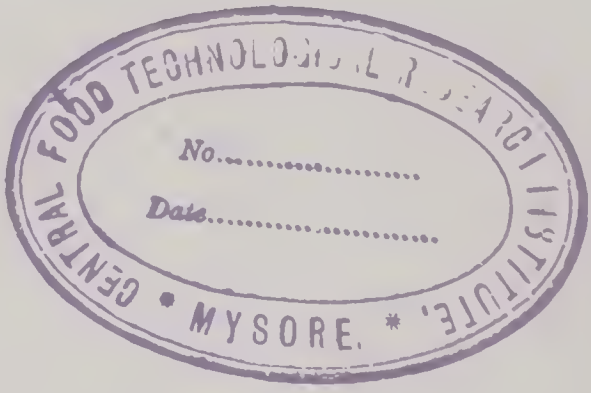
PROTEINS AND AMINO ACIDS

Physiology, Pathology, Therapeutics



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Preface

A large share of the responsibility for seeing that a people is well fed rests with the medical profession. That the importance of dietetics is generally recognized today is evidenced by the ever-growing volume of literature dealing with the various factors involved. Of these, vitamins and minerals certainly have received at least their fair amount of attention. And of the three basic foodstuffs — carbohydrates, proteins and fats — while all may serve to supply energy, only proteins can furnish nitrogenous material essential for tissue growth, maintenance and repair. Their singular importance is thus beyond refutation.

This book was prepared under the supervision of the Scientific Staff of The Arlington Chemical Company. Acknowledgment is gratefully made to the many outstanding workers in the field, conferences with whom helped to formulate many of the editorial opinions. None of these men is mentioned by name since responsibility for these opinions is wholly ours. Invaluable assistance in the compilation of the literature and preparation of the manuscript was afforded by Miss Doris Berlin, whose efforts make this work as comprehensive as it is.

While no pretense to completeness is made, it is believed that all of the available major scientific publications through June 1944 have been reviewed. Permission for the direct quotations used is gratefully acknowledged.

At a time when fundamentals are receiving merited attention in medicine, as well as in other spheres, it is believed that this handbook on the metabolism of proteins and amino acids will be of value to the busy clinician.

THE EDITORS

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PART ONE

NORMAL

PROTEIN METABOLISM

The human body has been likened to a house whose building stones are protein bricks. And although water is by far the principal constituent of this house, without a supporting framework to give shape, form and substance, no house could it be. Therefore, in coining the name, protein, for this structural organic necessity for the human home, Mulder, the Dutch chemist, made use of the Greek word, πρωτειος, meaning "of the first importance". Hardly more than a hundred years have passed since Mulder's recognition of the significance of proteins, yet innumerable researches, especially within the last twenty-five years, have amply confirmed his original evaluation of their predominance in and their necessity for life itself.

Analyses of proteins with regard to their constituent elements – carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, copper, iodine, iron – have revealed much valuable information, but the knowledge that some of these chemical elements unite to form compounds called amino acids has been of even greater significance to the medical sciences. Amino acids, then, are really the building stones of proteins just as proteins are the structural units of body tissues, and any discussion of proteins must of necessity involve a consideration of amino acids.

Section One . . . OCCURRENCE OF PROTEINS

All living things, plants and animals alike, contain proteins in their cellular protoplasm, cell wall and nuclear material. In the human body alone, Mathews *(1)* has estimated that there are about two hundred different kinds of cells. With each cell containing at least eight different proteins, there are, he calculates, about 1600 different proteins in each one of us! Yet the total weight of all these amounts to but an average of eleven kilograms per person.

A basic obstacle hindering the clarification and subsequent classification of the relationships and functions of all these body proteins is the inadequacy of our knowledge concerning their molecular structure. This, in turn, is due to the fact that proteins are among the largest molecules known. Contrast, for example, dextrose whose molecular weight is 180, with hemoglobin having a molecular weight of about 66,000, or serum globulin with a molecular weight of perhaps 175,000. The difficulties involved in assembling a concrete picture of the arrangement of the elements into amino-acid units, and the arrangement of the amino acids themselves in these huge molecules can thus be appreciated.

One of the fundamental differences between animals and plants rests in the ability of the latter to synthesize its components from the chemical elements. The wheat stalk manufactures its constituent proteins (*e.g.*, gliadin, glutenin and leucosin) from the nitrogen and sulfur of the soil, and the carbon, oxygen and hydrogen of the atmosphere. But man must ingest ready-made proteins which are then broken down and rearranged to suit his own need, for each species contains distinctive proteins in its tissues.

Since the body's source of proteins is the food ingested, the following points come to mind: Which food or classes of foods

are relatively rich in proteins? How much protein must be eaten to provide optimum health? What are the differences, if any, between the nutritive values of various foods with respect to their ability to satisfy protein requirements? It may be well to consider, first, protein needs and to follow this by a discussion of the relative values of different proteins and foods in fulfilling them.

REFERENCE

1. Mathews, A., Physiological Chemistry, Baltimore, Williams and Wilkins Co. (1939) p. 358

Section Two . . . PROTEIN REQUIREMENTS

It is apparent that no fixed amount of protein will serve to satisfy body needs for all stages of life. Growth, pregnancy and lactation obviously demand amounts far in excess of that required for maintenance. With regard to maintenance, estimates of the optimum daily intake have ranged from 118 grams (Voit in the last century) *(1)* to 44 grams (Chittenden in the early twentieth century) *(2)*. The prevailing consensus favors about one gram of protein per kilogram of body weight for normal adults.

Table I gives the standards set forth by the Committee on Food and Nutrition of the National Research Council. Use of this table as a guide to optimum nutrition implies an understanding on the part of the user of several pertinent facts.

For most of the dietary constituents listed, little or no data as to absolute human requirements are available. Amounts given represent what nutrition experts consider to be productive of optimum health on the basis of evidence presently available. The problem is complicated by the fact that requirements for any one substance may change when quantities of other substances with which it is metabolically associated are altered. Thus, for example, requirements for thiamine depend upon carbohydrate intake and utilization.

Irrespective of other factors, the situation with regard to the protein intake is, as we shall see, unique in that, of the dietary components listed, it has qualitative as well as quantitative aspects.

REFERENCES

1. Hermann, L., editor, Handbuch der Physiologie, Leipzig, F. C. Vogel (1881) Vol. 6
2. Chittenden, R. H., Physiological Economy in Nutrition, N. Y., Stokes and Co. (1904)

TABLE I

RECOMMENDED DIETARY ALLOWANCES*

National Research Council, Reprint and Circular Series Number 115, January, 1943.

	Calories	Protein grams	Calcium grams	Iron mg.	Vitamin A*** I. U.	Thiamine (B_1) mg. **	Ribo-flavin mg.	Niacin (Nicotinic Acid) mg.	Ascorbic Acid mg. **	Vitamin D I.U.
Man (70 Kg.)										
Sedentary	2500	....	.....	....	....	1.5	2.2	15	....	....
Moderately active	3000	70	0.8	12	5000	1.8	2.7	18	75	+++
Very active	4500	....	.....	....	....	2.3	3.3	23	....	....
Woman (56 Kg.)										
Sedentary	2100	....	.....	....	....	1.2	1.8	12	....	....
Moderately active	2500	60	0.8	12	5000	1.5	2.2	15	70	+++
Very active	3000	....	.....	....	....	1.8	2.7	18	....	....
Pregnancy (latter half)	2500	85	1.5	15	6000	1.8	2.5	18	100	400 to 800
Lactation	3000	100	2.0	15	8000	2.3	3.0	23	150	400 to 800
Children up to 12 years:										
Under 1 year +	100/Kg.	3 to 4/Kg.	1.0	6	1500	0.4	0.6	4	30	400 to 800
1–3 years ++	1200	40	1.0	7	2000	0.6	0.9	6	35	+++
4–6 years	1600	50	1.0	8	2500	0.8	1.2	8	50	....
7–9 years	2000	60	1.0	10	3500	1.0	1.5	10	60	....
10–12 years	2500	70	1.2	12	4500	1.2	1.8	12	75	....
Children over 12 years:										
Girls, 13–15 years	2800	80	1.3	15	5000	1.4	2.0	14	80	+++
16–20 years	2400	75	1.0	15	5000	1.2	1.8	12	80	....
Boys, 13–15 years	3200	85	1.4	15	5000	1.6	2.4	16	90	+++
16–20 years	3800	100	1.4	15	6000	2.0	3.0	20	100	....

* Tentative goal toward which to aim in planning practical dietaries; can be met by a good diet of natural foods. Such a diet will also provide other minerals and vitamins, the requirements for which are less well known.

** 1 mg. thiamine equals 333 I. U.; 1 mg. ascorbic acid equals 20 I. U.

*** Requirements may be less if provided as vitamin A; greater if provided chiefly as the provitamin carotene.

\+ Needs of infants increase from month to month. The amounts given are for approximately 6–8 months. The amounts of protein and calcium needed are less if derived from human milk.

++ Allowances are based on needs for the middle year in each group (as 2, 5, 8, etc.) and for moderate activity.

+++ Vitamin D is undoubtedly necessary for older children and adults. When not available from sunshine, it should be provided probably up to the minimum amounts recommended for infants.

The requirement for iodine is small; probably about 0.002 to 0.004 milligram a day for each kilogram of body weight. This amounts to about 0.15 to 0.30 milligram daily for the adult. This need is easily met by the regular use of iodized salt; its use is especially important in adolescence and pregnancy.

The requirement for copper for adults is in the neighborhood of 1.0 to 2.0 milligrams a day. Infants and children require approximately 0.05 per kilogram of body weight. The requirement for copper is approximately one-tenth of that for iron.

The requirement for vitamin K is usually satisfied by any good diet. Special consideration needs to be given to newborn infants. Physicians commonly give vitamin K either to the mother before delivery or to the infants immediately after birth.

Section Three . . . NITROGEN BALANCE

In determining the protein requirements of adults, use has been made of nitrogen balance determinations. Since their nitrogen content is particularly characteristic of proteins, information concerning protein metabolism may be derived from analyses for nitrogen in foodstuffs and excreta by using an appropriate conversion factor. (Since many proteins contain about 16% nitrogen, it has become a convention to convert percent nitrogen to percent protein by multiplying by 6.25.)

Nitrogen balance occurs when the nitrogen of the diet equals the nitrogen of the urine plus the small loss in the feces. When the urinary nitrogen exceeds dietary nitrogen, as during fevers, wasting disease and inadequate protein consumption, then a state of negative nitrogen balance exists. Conversely, a positive nitrogen balance (a retention of nitrogen evidenced by greater amounts of dietary than urinary nitrogen) normally occurs during periods of increased need for protein as in growth, pregnancy or convalescence. The amount of protein which just suffices to keep an individual in nitrogen equilibrium, that is, the amount which supplies enough nitrogen to cover that lost in the urine, satisfies minimum requirements and provides for no more than maintenance of the status quo. To allow growth or tissue repair after injury to proceed, a state of positive nitrogen balance must be attained.

From the above, it would appear that the nitrogen requirement of an individual is simply a quantitative one, and that any food containing a sufficient amount of protein would satisfactorily fulfill the need. The general reliance upon meat (rich in protein as determined by its nitrogen content) rather than on vegetables, cereals or legumes for the additional nitrogen intake of a high protein diet would seem to bear this out. Yet a consideration of the following suggests otherwise. Laboratory

analyses show that milk contains 3.3% proteins; pecans, 11.28%; and gelatin, 91.4% *(1; 2)*. Of these three sources of dietary protein, however, milk is the only one which by itself can satisfy the nitrogen requirements of the human. In the case of the nut, its inadequacy, in large part, is due to its lower digestibility (71% as contrasted with 98% for milk or milk products) *(2)*. Thus, one factor determining the nutritive value of a protein is its digestibility – and the concomitant availability of its amino acids from the gastrointestinal tract. (Further reference to the availability of food proteins is included in the discussion of digestion.)

That quality of protein is also an important factor was discovered some time ago when, in the course of nitrogen balance studies, it was observed that proteins fed at the same level of intake (corrected for digestibility) varied widely in their ability to provide nitrogen equilibrium and growth. One measure of these differences, known as the 'biological value' of a protein, is defined by Mitchell *(3)* as the percentage of absorbed nitrogen (nitrogen intake minus fecal nitrogen of dietary origin) not eliminated in the urine. This is an index of the ability of proteins to fulfill growth requirements, since the greater the percentage retained, the greater will be the increase in weight. According to this standard, gelatin has no 'biological value' whatsoever since it fails to provide nitrogen equilibrium and thus cannot satisfy even maintenance requirements, regardless of the quantity ingested *(4)*. Milk proteins, on the other hand, have high 'biological value' (85%).

The determination of 'biological values' serves as a means of evaluating proteins with regard to support of growth. In order to explain differences in 'biological values', however, reference must be made to the amino acid composition of proteins.

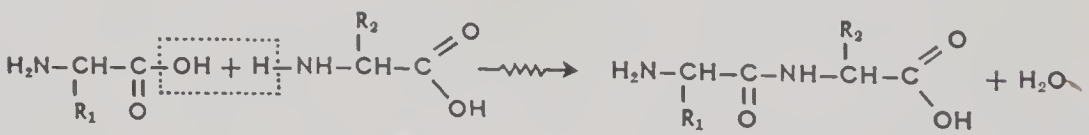
REFERENCES

1. Bridges, M. A., Dietetics for the Clinician, Phil., Lea and Febiger (1941) p. 681
2. Mitchell, H. H. and Beadles, J. R., J. Nutrition. *14*,597 (1937)
3. Mitchell, H. H., Physiol. Revs., *4*,424 (1924)
4. Mitchell, H. H. and Hamilton, T. S., The Biochemistry of the Amino Acids, N. Y., Chemical Catalog Co. (1929) p. 254

Section Four . . . AMINO ACIDS AS COMPONENTS OF PROTEINS

Hydrolysis of proteins by means of acids, alkalies or enzymes results in disappearance of the colloidal character of the original protein and liberation of crystallizable substances which can be separated from each other. These crystallizable amino acids have been extensively studied so that at the present time the structure and properties of over 40 of them are known *(1)*. Chemically speaking, those amino acids with which the physician is concerned are organic acids possessing an amino (NH_2) (or imino [NH] in the case of proline and hydroxyproline) group in place of one of the hydrogens attached to the alpha carbon atom, that is, the carbon next to the carboxyl (COOH) radical. Thus their general formula is R.CH (NH_2) COOH. In the nutritional sense, the use of the term is confined to amino acids found in food proteins. The names and structural formulas of the most important of these will be found in Table II.

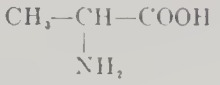
Our knowledge of the manner in which amino acids are linked within the protein molecule is based upon the pioneer researches of Emil Fischer. By coupling amino acids into peptides by more or less complex chemical processes which may be schematically reduced to the following example:



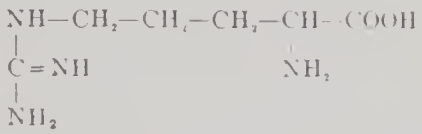
he showed how long chains (polypeptides) might be built up and further demonstrated certain formal analogies of behavior between these synthetic peptide models and naturally occurring proteins. The peptide linkage (-CONH-) between amino acids may be considered one of the principal 'cementing' materials for these protein-building bricks.

TABLE II — *Amino Acids*

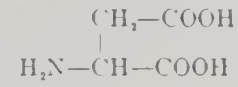
1. Alanine



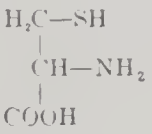
2. Arginine



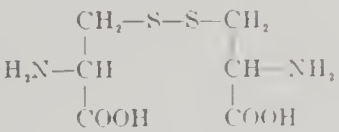
3. Aspartic Acid



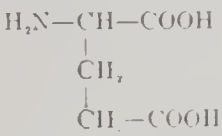
4. Cysteine



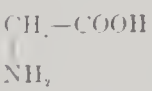
5. Cystine



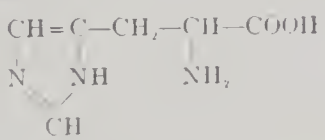
6. Glutamic Acid



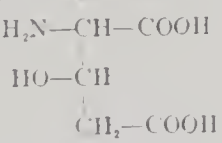
7. Glycine (Aminoacetic Acid)



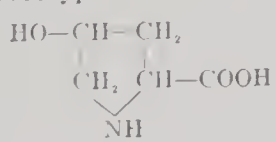
8. Histidine



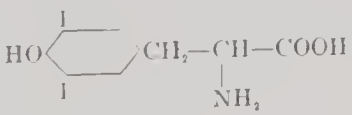
9. Hydroxyglutamic Acid



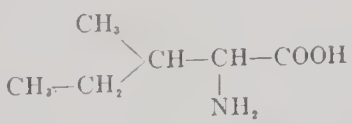
10. Hydroxyproline



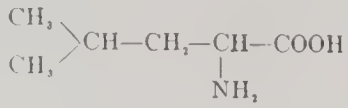
11. Iodogorgoic Acid (Diodotyrosine)



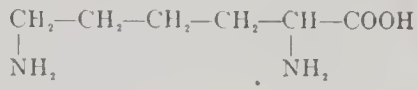
12. Isoleucine



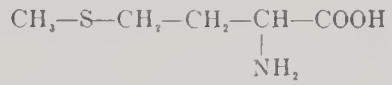
13. Leucine



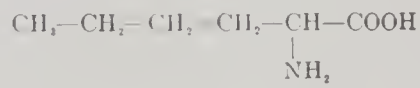
14. Lysine



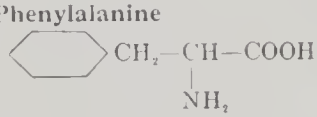
15. Methionine



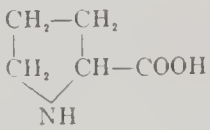
16. Norleucine



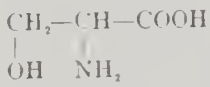
17. Phenylalanine



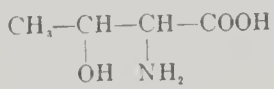
18. Proline



19. Serine



20. Threonine



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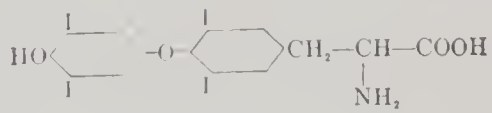

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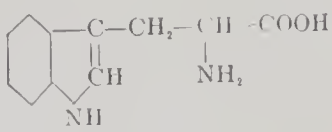

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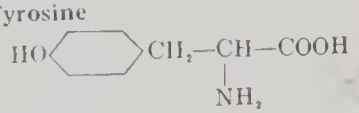

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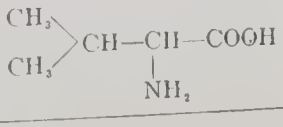

All of the known amino acids except glycine are optically active by virtue of the presence in their structure of one or more asymmetric centers (carbon atoms each of whose four valences are linked to different chemical structures). The synthesis of such compounds in the laboratory gives rise to at least two individuals chemically indistinguishable but differing in important physical and biological properties.

In 1906, Willcock and Hopkins *(3)* and in 1914, Osborne and Mendel *(4)* reported the results of their experiments in maintaining rats upon synthetic diets containing a single protein known to be deficient in one or more amino acids as the sole source of nitrogen in the diet. It was found that nitrogen balance could not be attained and that the animals could not grow normally on diets whose nitrogen was supplied by zein, the chief protein of maize. The addition of lysine and tryptophan to the ration enabled the animals to grow. It was thus established that, for normal growth to proceed, certain amino acids were required in the diet. These amino acids which the body apparently cannot synthesize for this purpose are called 'essential'.

Classification of amino acids on the above basis has been extended by Rose and his collaborators. These investigators employed synthetic diets, the nitrogen of which was furnished by mixtures of crystalline amino acids. At first they found that a mixture of all the then-known amino acids failed to provide nitrogen equilibrium. It was reasoned that one or more unknown amino acids were lacking, and studies along this line very shortly led to the discovery of the amino acid, threonine, which, when added to the mixture of crystalline compounds, provided positive nitrogen balance and growth *(5; 6)*. By this experiment both the essential nature of a hitherto unknown amino acid was discovered, and a dietary tool in the form of a mixture of synthetic amino acids adequate for growth and maintenance was secured. Continuing his research by altering

the various components of this mixture, Rose *(7)* was finally able to classify the amino acids with respect to their necessity for growth in the rat. (Table III)

TABLE III

Classification of Amino Acids with Respect to Their Growth Effects in Rats

Essential	*Non-Essential*
Arginine	Alanine
Histidine	Aspartic Acid
Isoleucine	Citrulline
Leucine	Cystine
Lysine	Glutamic Acid
Methionine	Glycine
Phenylalanine	Hydroxyglutamic Acid
Threonine	Hydroxyproline
Tryptophan	Norleucine
Valine	Proline
	Serine
	Tyrosine

The relative nutritive values of the optical antipodes of the essential amino acids (except arginine) have been determined; only the naturally occurring isomeric forms of valine, leucine, isoleucine, lysine and threonine are effective in the animal organism (rat), whereas both isomers of tryptophan, phenylalanine, methionine and histidine are utilized *(2)*.

Although Rose *(2)* claimed that rats whose dietary nitrogen was derived exclusively from a mixture of the ten 'essential' amino acids "gained in weight just as rapidly as when all of the protein components were supplied preformed", Albanese and Irby *(8)* have presented evidence that a mixture of the 'essential' amino acids is nutritionally inferior to enzymatic or fortified acid casein hydrolysates or

casein itself. Rose *(9)* has recently written that later experiments involving a large number of animals have shown that mixtures of 19 amino acids give slightly better growth response than mixtures of the 10 'essential' amino acids; animals on the latter diet, however, in no case lost weight.

In Albanese's experiments, with feedings restricted to comparable levels, positive nitrogen balance was maintained with both the amino acid mixture and the fortified acid casein hydrolysate. (It should be mentioned that all of the diets contained protein from brewer's yeast to the extent of about 10% of the total protein.) However, while the fortified acid casein hydrolysate diet allowed continued normal weight gain, the 'essential' amino acid mixture diet was accompanied by a marked weight loss. The amount of isoleucine in the mixture is not stated; it may have been insufficient.

It is somewhat difficult to understand weight loss in the presence of a favorable nitrogen balance and an otherwise adequate diet unless the dietary nitrogen is derived from non-protein sources. Possibly the divergence between the reports of Rose and Albanese is to be explained by the absence of some unrecognized growth factor in the amino acid diet. This factor would not, then, be supplied with the particular brewer's yeast offered as a vitamin source in Albanese's experiments but might be present in the variously hydrolyzed or intact casein preparations. The nature of the vitamin supplement in Rose's experiments has not been described.

Further, it is difficult to reconcile these experimental results with those of Whipple and his co-workers *(10)* who found a mixture of the ten 'essential' crystalline amino acids a highly satisfactory source of nitrogen nutrient in anemic and hypoproteinemic dogs, giving positive nitrogen balance and a good regeneration of hemoglobin and plasma proteins with no evidence, in these highly susceptible animals, of toxic effects due to the unnatural isomers of some of the amino acids. Elman and Lischer *(11)* found no indication of toxicity in their studies of amino acid mixtures in dogs in experimental hemorrhagic shock. Albanese and Irby hypothecate toxicity of unutilizable unnatural isomers of certain amino acids as a possible explanation of their experimental results. Kinsey and Grant *(11a)* have reported no toxic effects from unnatural amino acid isomers in experiments on rats. Their conclusions confirm Rose's findings.

Bearing in mind the uncertainty of comparing the reactions between different species or the divergence between the requirements for growth in one species and of maintenance in a second species after an artificial pathological condition has been induced, extreme caution should attend the drawing of any conclusions from these experiments as to the sufficiency of a diet most of whose nitrogen derives from a mixture of the 'essential' amino acids in experimental animals.

It should be emphasized that Rose's classification refers to the requirements of one species, the rat, for one physiological function, growth. Almquist *(12)* has shown that the chick differs distinctly from the rat as concerns the essential nature of glycine. In the dog, however, those amino acids which are dispensable are the same as those dispensable in the rat *(13)*. Some experiments have been reported which suggest that threonine may not be 'essential' for canine nitrogen balance *(14)*. More recently it has been shown in intravenous tests that dogs may be kept in positive nitrogen balance on a mixture of eight amino acids (all of the 'essentials' except histidine and arginine) for not more than three days *(15)*. Acid casein hydrolysates, deficient in tryptophan, reduced the urinary nitrogen excretion from that observed with the nitrogen free diet but did not allow positive nitrogen balance to be attained. The importance of the duration of the experiment on the conclusion to be drawn is thus emphasized.

Present investigations have as their objective the determination of human requirements. So far, a mixture of eight amino acids – all of those described as essential for growth in rats except arginine and histidine – has been found necessary for the maintenance of nitrogen balance in normal young adults *(16; cf. 17; 18-21)*.

The relative or absolute quantitative requirements of any species in terms of a mixture of the 'essential' amino acids will have only limited value, since these requirements will probably undergo sub-

stantial changes, relative to each other and absolutely, when the 'non-essential' amino acids are included in the diet in variable makeup and quantity.

Another factor which has been largely overlooked is the possibility of the supply, in all or part, of one or more of the 'essential' amino acids by bacterial synthesis in the intestinal tract. This concept has been mentioned by Rose and made reasonable by the recent studies on the amino acid requirements of several common bacterial species, *e.g.*, Lactobacilli. (Also see *21a.*)

Since the formation of any protein requires varying quantities of all of the amino acids, restriction of the diet to only the 'essentials' must add considerably to the burden placed on the synthetic functions of the body, all of the 'non-essentials' having to be manufactured from the 'essentials' supplied. While such a restricted amino acid intake might be used effectively under certain limiting conditions presenting special problems, it may not reproduce the normal course of protein metabolism.

Knowledge concerning the amino acids necessary for a particular process cannot be too freely extended to imply their necessity for other functions in the same species. Thus, arginine is synthesized by the rat at a rate barely sufficient for maintenance, so although listed as essential for growth, it is dispensable for the former process; it has been demonstrated that adult female rats may be maintained in nitrogen equilibrium on but seven of the ten 'essential' amino acids *(2; 22)*. Ultimately we may be able to distinguish between the amino acids required for growth, for example, from those required for maintenance or lactation, but at present proteins are classed as 'complete' or 'incomplete' depending upon the mere presence of the 'essential' amino acids.

Table IV is a compilation from various sources of the amino acid composition of different proteins *(23; 24)*. In general it may be said that cereal and vegetable proteins are relatively deficient in lysine, which fact may account for their lower

TABLE IV—*Amino Acid Composition of Proteins*†

	Alanine	Arginine	Aspartic Acid	Cystine	Glutamic Acid	Glycine	Histidine	Hydroxy-glutamic Acid	Hydroxy-proline	Leucine	Lysine	Methionine	Phenylalanine	Proline	Serine	Tryptophane	Tyrosine	Valine	Ammonia	Total **
Casein	1.9	3.8	4.1	0.3	21.8	0.5	2.5	10.5	0.2	9.7	6.0	3.4	3.9	9.0	0.5	2.2	6.6	7.9	2.3	97.1
Lactalbumin	2.4	3.0	9.3	4.3	12.9	0.4	2.1	10.0		14.0	8.8	2.6	1.3	3.8	1.8	2.7	2.0	3.3	1.3	86.0
Egg Albumin	2.2	5.2	6.1	1.3	14.0	0	1.4	1.4		10.7	6.4	4.6	5.1	4.2		1.3	4.2	2.5	1.4	72.0
Egg Vitellin	0.2	7.8	0.5	1.2	12.2	1.1	1.2			11.0	5.4		2.8	4.0		1.3	5.0	2.4		56.1
Wheat Gluten	0.3	4.4	0.7		24.0	0.4	1.2			4.1	2.2		1.0	4.0			1.9			44.2
Wheat Gliadin	2.0	2.9	0.8		43.7	0	1.5	2.4		6.6	0.6	2.1	2.4	13.2		1.1		3.4	5.2	87.9
Wheat Glutenin	4.7	4.7	2.0	1.8	25.7	0.9	1.8	1.8		6.0	1.9		2.0	4.2	0.7	1.7	4.5	0.2	4.0	68.6
Wheat Leucosin	4.4	5.9	3.3	1.5	6.7	0.9	2.8			11.3	2.7		3.8	3.2		1.2	3.3	0.2	1.4	52.6
Beef Muscle Protein*	3.7	7.5	5.9	1.55	15.5	2.1	1.76			11.6	7.6		3.2	5.8		1.25	2.2	0.81	1.07	71.54
Chicken Muscle Protein*	2.3	6.5	3.2	0.64	16.5	0.68	2.5			11.2	7.2		3.5	4.7		P	2.2		1.67	62.79
Gelatin	8.7	8.2	3.4	0.2	5.8	25.5	1.0	0	14.1	7.1	5.9	0	1.4	9.5	0.4	0	0	0	0.4	91.6
Pea Legumin	2.1	11.7	5.3	0.9	17.0	0.38	1.69			8.0	5.0		3.8	3.2	0.53	1.76	1.55		2.0	64.91
Barley Hordein	0.43	2.9		1.55	43.2	0	2.1			5.7	1.01		5.0	13.7		1.05	1.67	0.13	4.8	83.24
Zein	9.8	1.8	1.8	1.0	31.3	0	0.8	2.5	?	25.0	0	P	7.6	9.0	1.0	0	5.9	1.9	3.6	103.0
Serum Albumin	2.7	4.8	3.1	6.1	7.7	0	1.2			20.0	6.9		3.1	1.0	0.6	0.5	4.7			62.4
Serum Globulin	2.2	5.2	2.5	1.0	8.2	3.5	0.9			18.7	6.2		3.8	2.8		2.3	6.7			64.0
Hemoglobin	4.2	3.1	4.4	0.3	1.7		7.6		1.0	29.0	8.1		4.2	2.3	0.6	1.3	3.2			71.0
Histone (Thymus)	3.5	27.1			0.5	0.5	5.8			11.8	9.7		2.2	1.5			5.2		7.5	75.3
Keratin (Hair)	1.5	8.0		18.9		4.7	0.5			7.1	2.5			3.4		1.8	4.8	1.0		54.2
Pepsin		2.7	6.8	1.4	18.6		0.1				2.1					2.2	10.3		8.8	53.0
Insulin		3.0		12.0	21.0		8.0			30.0	2.0						12.0			88.0
Bence-Jones Protein	4.5	5.1	2.2	3.0	8.0	1.7	1.1			10.6	6.8		4.9	2.7		2.5	6.8	5.6	5.6	71.1

*—not a single protein. P—present.

**—because of the introduction of one molecule of H_2O for each peptide linkage broken, the theoretical summation should be approximately 115% to 125% for each of these proteins.

†—analytical data for isoleucine and threonine are omitted because of the paucity of available figures.

nutritive values. The completeness of milk as a protein food is seen in the amino acid content of both casein and lactalbumin. Of particular interest to the clinician are the analyses of hemoglobin, serum albumin and globulin, pepsin and insulin.

"In practical dietetics neither animals nor man are ever restricted to a single protein as a source of the necessary amino acids" *(25)* – hence the significance of the supplementary effects of proteins. Clearly, the biological value of any mixture of two or more proteins will never be less than the weighted mean of the values for the individual proteins, while very often it has been found to exceed this figure.

If the amino acid or acids (tryptophan and lysine, for example) lacking in the first protein, zein in this case, are not the limiting factors for the second protein, lactalbumin (in other words, if both proteins are not deficient in the same amino acids), then, in a mixture of the two proteins, the first will not only be enhanced by, but will also itself enhance the second. The addition of lactalbumin to zein provides a complete assortment of amino acids for growth. The addition of 13.5% zein to only 4.5% lactalbumin more than doubles the growth rate secured from the latter alone *(4)*.

This ability of incomplete proteins to be utilized so effectively in supplementing complete proteins suggests the value of gelatin, for instance, in diets containing complete proteins as well. The mechanism by which supplementation is effective undoubtedly lies in the presentation to the body of a more complete supply of amino acids. Since vegetable proteins are generally deficient in lysine and animal proteins are usually richer in their content of this amino acid, it follows that animal proteins are better supplements for vegetable proteins than are other vegetable proteins. (*cf.* p. 159)

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18 . . .

Section Five . . . ALIMENTARY TRACT PROCESSES

As stated by White *(1)*, "The primary function of digestion is to break down complex, nondiffusible molecules to simple diffusible compounds and ions". In the case of proteins, this is accomplished by the successive action of gastric, pancreatic and intestinal enzymes; and the simple diffusible compounds resulting therefrom are mainly amino acids.

When ingested protein is exposed to the action of gastric juice, the proteolytic enzyme, pepsin, hydrolyzes the molecule to proteoses and peptones. The possibility that amino acids may be set free during peptic digestion, especially if this proceeds for a sufficient length of time, has been demonstrated *(2; 3)*. However, the usual propulsion of the food bolus into the duodenum within four hours makes it seem unlikely that this takes place to any extent under normal circumstances. Rennin, the second proteolytic enzyme of the stomach, appears to be of particular importance in infant nutrition, but since it has been proven that pepsin and trypsin both possess milk-curdling properties, the importance of rennin in adult nutrition may be questioned.

Food reaching the intestine is acted upon by the three proteolytic enzymes of the pancreatic juice as follows: trypsin and chymotrypsin liberate free amino acids from native proteins; and carboxypolypeptidase acts on polypeptide intermediates to yield the same end products. Similar in action to carboxypolypeptidase are the two enzymes of the intestinal juice, or succus entericus, formerly considered to be a single entity called erepsin. In the small intestine, the final breakdown of protein is accomplished; here, too, absorption of the liberated amino acids takes place. Physiological evidence for this is furnished by a progressively increasing concentration of amino acids in the portal blood, the liver and the systemic circulation

following their appearance in the intestinal tract *(4; 5)*. Most of the nitrogen of a 4% to 5% solution of gelatin or casein, introduced directly into the jejunum, is absorbed within 40 to 50 minutes, while the nitrogen of a mixture of amino acids (protein hydrolysate) introduced in the same manner practically disappears completely in 15 to 25 minutes *(6)*. That larger molecules such as polypeptides may also diffuse through the wall of the intestine was suggested by London and Kotschneff *(7)*. Possibly though, the observed increase in polypeptide concentration in portal blood was due to a synthesis from amino acids during the passage of the latter through the intestinal wall *(8)*.

A discussion of the fate of proteins in the alimentary tract would be incomplete without reference to undigested protein as well as to the protein utilized by bacteria. In the case of the former, an index known as the 'coefficient of digestibility of protein' is computed from the amounts of ingested and fecal nitrogen. The coefficient represents the percentage of intake which is absorbed by the body. When it is desired to secure the same level of nitrogen intake with different proteins, their several digestibility coefficients must be taken into account. That these differences may be considerable is shown by the following coefficients: meat, 97%; cereals, 85%; legumes, 78%; fruits, 85%; vegetables, 83% *(9)*. It is likely these figures are no more than 'apparent' digestibilities since fecal nitrogen, in addition to containing undigested food nitrogen, also consists of nitrogen from dead bacterial and epithelial cells, as well as nitrogen from the digestive juices *(10)*.

Intestinal putrefaction results from bacterial action on protein. One of the possible reactions involves splitting off of the carboxyl radical of an amino acid, with the production of the related amine, *e.g.* — tyramine from tyrosine, histamine from histidine, etc. From tryptophan are formed indole and skatole,

which impart to the feces their characteristic odor. A portion of the indole is absorbed and subsequently eliminated in the urine, so that urinary indican determinations may be used to reveal the extent of intestinal putrefaction. Another chemical reaction which may take place in consequence of the simultaneous presence of bacteria and protein in the intestine is deamination, the removal of an NH_2 group, with the production of ammonia and other compounds such as fatty acids, hydroxy-acids, phenols, and alcohols. Since the energy requirements of microbes may be supplied by either carbohydrates or protein, and since bacteria have been shown to prefer carbohydrates when given access to both, inhibition of intestinal putrefaction may be accomplished by the feeding of non-digestible carbohydrate.

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Section Six . . . UTILIZATION OF PROTEIN FOR ENERGY

Deamination and Urea Formation

After passing through the intestinal wall and entering into the blood stream, the amino acids are carried to the liver via the portal vein. Some continue into the systemic circulation, where they are rapidly taken up by the body tissues. Within the liver, another part of the amino acids undergoes deamination, leading to the liberation of ammonia and a non-nitrogenous residue, the 'carbohydrate moiety' *(1)*. (For the essential role of the liver in the deamination reaction, see *(2)*.) The ammonia is excreted as urea, into which it may be converted by a series of reactions following its combination with carbonic acid. Evidence has been advanced showing the splitting of urea from arginine by means of the enzyme, arginase *(3)*. Ornithine, also formed from this reaction, unites with ammonia and carbon dioxide through the intermediary, citrulline, to yield arginine again; thus the reaction is cyclic. Recent evidence *(4; 5)* tending to exclude the participation of arginase has been offered.

There are several possible paths which the carbohydrate moiety, the keto-acid remaining after deamination, may pursue. It may be reaminated; it may be oxidized directly to carbon dioxide and water for fuel; it may be converted to glucose (and stored as glycogen) or to fat. Not all the deaminated amino acids are sugar-formers; some of them yield fatty acids and acetone, while others such as tryptophan, lysine and histidine are neither glycogenic nor ketogenic. In its aggregate influence on metabolism, protein is considered to be antiketogenic *(6)*. Studies on diabetic animals have revealed that as much as 60 grams of glucose can be produced from 100 grams of protein *(7)*.

Protein-sparing Effect of Carbohydrates

The extent to which protein is utilized as a source of energy depends to a considerable degree upon the proportionate amounts of non-nitrogenous nutrients simultaneously ingested. (Other factors are the momentary energy and nitrogen needs of the body, and the suitability of the assortment of amino acids offered to fulfill the nitrogen requirements.) The greater the amount of carbohydrate, particularly, the smaller is the excretion of urinary nitrogen, indicating greater utilization of the amino acids for their more exclusive functions in the body. This protein-sparing effect of carbohydrates *(8)* is of importance in attaining the most efficient use of ingested protein. McLester *(9)* says that for nitrogen economy, 50% to 60% of the daily caloric intake should be supplied by carbohydrates; calculated on a weight basis, this is equivalent to 4 to 5 times as much carbohydrate as protein.

Specific Dynamic Action of Proteins

The increase in basal metabolic rate due to ingestion of a foodstuff often is observed to exceed the intrinsic caloric value of the foodstuff. This stimulus to tissue activity is called specific dynamic action. Protein ingestion gives a specific dynamic effect equivalent to 30% of its caloric value, while the corresponding figures for carbohydrates and fats are but 6% and 4% respectively. It follows, then, that a high protein diet will dissipate a greater amount of energy than an isocaloric diet containing less protein and more of the other two foodstuffs. The process through which nitrogenous food exerts this marked increase in metabolism has been shown to take place in the liver *(10)*. It may be related to the deamination of certain amino acids, notably glycine, alanine, leucine, glutamic acid, tyrosine, and phenylalanine. The specific dynamic effect following meat ingestion has been explained from this

viewpoint *(11)*. Whether the fundamental details be known or not, a useful application of the phenomenon is seen in the high protein diets prescribed for the treatment of obesity.

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Section Seven . . . EXOGENOUS AND ENDOGENOUS METABOLISM

Folin's classical studies *(1)* led him to conclude that protein metabolism is of two kinds, exogenous and endogenous. The former, extremely variable in quantity, is applied to the metabolism of ingested protein; the latter, a constant, relates to tissue protein breakdown and is measured in any individual largely by the creatinine excretion. The amount of this endogenous metabolite normally appearing in the urine indicates that only a small part of the protein of an individual is hydrolyzed daily. Urea excretion may be due to endogenous as well as exogenous metabolism, for Schoenheimer and his colleagues *(2)* have shown that a continuous breakdown and replacement of tissue protein takes place, with similar intermediate reactions such as deamination and urea formation occurring as in the breakdown or catabolism of ingested protein.

Mitchell *(3)* recently reasserted the independence of these two forms of metabolism. Subsequently he deduced *(4)* that Schoenheimer's concept of dynamic interchange introduces no incompatabilities. On the other hand, extensive experiments by Whipple and his associates have led them to the conclusion that differentiation of metabolism into exogenous and endogenous is essentially artificial. Their conclusions *(5)*, based largely on feeding experiments with dogs, are well supported by the elegant studies of Schoenheimer with the isotopically marked nutrients.

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Section Eight . . . ANABOLISM OF PROTEINS

In contrast to the sum total of knowledge concerning the catabolism or breakdown of amino acids, comparatively little is known about the anabolic processes through which dietary nitrogen is synthesized into body protein. We do know that, following the entry of amino acids into the portal vein, their concentration in the blood rises, but soon thereafter returns to normal levels in consequence of their rapid removal by body tissues, the liver especially *(1)*. In general, the amino acid concentration of the tissues is about ten times as great as that of blood plasma, even during fasting, and it is within the tissues that synthesis of the nitrogenous constituents of the body takes place, the processes being regulated by enzymes called intracellular proteinases *(2; 3)*.

Synthesis of Body Proteins

One of the most important intracellular anabolic reactions involves selection of the required amino acids for constructing the fixed body proteins characteristic of the individual. Mention must be made here of the endocrine factor and its influence upon the rate of this synthesis – especially the scarcely understood mechanisms of the anterior pituitary hormones *(4-6)*. Their stimulating role assumes particular prominence during growth, pregnancy and lactation – hence the basic and uncompromising need for adequate supplies of all the necessary amino acids during such periods, as well as when no accessory demands are made on the body.

With the attainment of adulthood (cessation of growth), maintenance rather than an increase in tissue mass is indicated. Obviously the protein requirement for this stage of life is lower than during growth, yet it is not as low as one might surmise because of a constant erosion or 'wear and tear' of body tissue.

That continuous chemical processes take place in tissue proteins even under conditions of nitrogen balance, and that dietary nitrogen enters the pool of these reactions tending to the ultimate replacement of tissue nitrogen has been revealed by the studies of Schoenheimer and his co-workers *(7)*, who utilized amino acids tagged by having been synthesized with isotopic (heavy) nitrogen (atomic weight 15 instead of 14). The heavier mass of the isotopic element permits its detection in the tissues; in this way the fate of ingested amino acids can be traced. The dynamic equilibria between tissues and their surrounding nutrient represent continuous metabolic processes, anabolic as well as catabolic in character. An as-yet-unknown mechanism balances these processes so that total body weight and tissue composition remain unchanged *(8)*.

Although less directly connected with nitrogen metabolism, it is germane to note that other physiological substances (iron and phosphorus compounds) which enter into proteins have been tagged by being rendered radioactive, and tracing these substances has given evidence of their dynamic metabolism. Similar evidence has pointed to the existence of such dynamic interchanges in fat metabolism as well, from which it seems reasonable to infer that there is nothing unique in the normal dynamic changes in the body proteins.

Superimposed on these normally occurring dynamic interchanges not revealed by weight measurements is the increased tissue catabolism resulting from wounds, fractures, fevers, ulcers, burns, etc. For example, one fracture case reported by Cuthbertson *(9)* lost 137 grams of nitrogen (856 grams of protein or nearly 10 pounds of tissue) in ten days. Losses such as this naturally increase the nitrogen requirements for maintenance of tissue mass.

Plasma Protein Synthesis

When laboratory determinations of blood plasma protein concentration are made, the figure obtained represents the sum of the concentrations of albumin and globulin. Properly speaking, the terms refer to at least six proteins – two albumins, three globulins, and fibrinogen (a globulin possessing distinctive chemical and physical properties). Prothrombin was formerly considered to be a globulin, yet more recent evidence places it among the albumins *(10)*. For clinical purposes, the albumins and globulins are considered single entities, each group manifesting different precipitation reactions upon addition of sodium sulfate solution *(11)*.

The site of formation of plasma proteins has been a debated issue, but certain general facts are relatively indisputable and may be considered as established. First, the liver has emerged as the organ primarily involved in plasma protein synthesis. With respect to fibrinogen production, the liver has been shown to be wholly responsible; fibrinogen synthesis following experimental hepatectomy or liver damage is markedly decreased *(12; 13)*. Conversely, injury to other tissues causes an elevation of blood fibrinogen *(14)*.

Considerable evidence points to the liver, also, as the locus of formation of albumin and globulin. A helpful tool for experimental work on this problem is the Eck fistula dog, that is, a dog whose portal vein is connected directly with the vena cava by surgical intervention, thus diverting from the liver blood coming to it from the intestines. When fed various food proteins as supplements to their basal diet, such dogs possess one tenth the capacity of normal dogs, fed similar diets, to produce plasma protein *(15)*.

The hypoproteinemia consequent upon cirrhosis of the liver is a clinical manifestation of the predominant role of this or-

gan in fabricating plasma proteins. Studies of the role of the reticulo-endothelial system in the manufacture of antibodies *(16)* suggest strongly that these tissues also participate in the synthesis of plasma protein, particularly globulins, with which fraction antibodies are associated. (See p. 42.)

In the formation of plasma protein, nitrogen from exogenous or endogenous sources, or, under certain conditions, both, may be utilized. Experimental studies have been confined to investigations of exogenous or endogenous nitrogen as isolated factors, insofar as has been possible.

With exogenous — dietary — protein, both quantity and quality are influencing factors on plasma protein synthesis. To study the comparative values of different proteins, two methods have been employed for rendering experimental animals hypoproteinemic: plasmapheresis and depletion by means of low protein diets.

Dogs are well suited for the induction of hypoproteinemia by plasmapheresis (exsanguination followed by reinjection of the washed red blood cells in physiological salt solution) *(17)*. Subsequent bleedings at frequent intervals maintain an approximately constant plasma protein concentration just above the edema level, the amount of plasma protein removed becoming an index of the potency of dietary nitrogen in its formation. Such dogs are clinically normal *(17a)*, except for low blood protein levels, and may be maintained in this condition for over a year as virtual biologic testing machines. In such experiments, carried out by Whipple and his associates *(18)*, the relative potency of different food proteins added to the basal diet given these animals, expressed as the number of grams required to produce one gram of plasma protein, varies widely: *e.g.*, beef serum, 3; salmon bread, 4; liver, 6; egg white, 5; casein, 8. In general, vegetable proteins favor the

production of globulin rather than albumin, though soy bean protein, like meat, favors albumin synthesis.

Substantially in agreement with Whipple's conclusions concerning food potencies in plasma protein fabrication are the results of the second method of approach employed by Weech and co-workers, a technique which perhaps more closely represents one of the more common processes of protein depletion in humans. These investigators *(19)* produced hypoproteinemia in their animals by administering diets low in protein for a period of three weeks. The rise of the serum albumin in the fourth week when the test protein is added to the basal ration is considered the 'assay value' of the test material. The average of a number of 'assay values' for a given protein, obtained from the same experimental procedure in several dogs, is called its 'potency value' for stimulating plasma protein regeneration *(20)*. Beef serum heads the list, with a 'potency value' of .801, followed by egg white (.616), milk (.483) and casein (.425).

Despite the similarities in the relative order of potency obtained by both groups of investigators, it must be kept in mind that the first-described technique measures plasma protein formation whereas the latter method uses the rate of albumin production as the basis for determining potency values. Furthermore, and more important, opposite conclusions concerning globulin regeneration are arrived at: Weech and his group *(19)* state that diet is not an important factor influencing the production of globulin; Madden and Whipple *(18)* found that, "as measured by plasmapheresis, diet regulates globulin production equally as well as albumin formation".

The use of endogenous nitrogen for plasma protein synthesis may be considered with reference to two types of tissue protein, the fixed cell proteins, and a more labile portion

called the reserve store, also definable as "all protein which may be given up by an organ or tissue under uniform conditions without interfering with organ or body function" *(18)*. First to observe the existence of the reserve store was Morawitz *(21)* in 1906, while later evidence was supplied by Whipple *(22; 23)* in 1919 and 1920, at which times regeneration of plasma proteins in the fasting dog following rapid depletion was described. In their review of the entire problem, Madden and Whipple indicate the extent of the reserve store by stating that it may replace 40% to 50% of the circulating plasma protein.

Reliable determinations of the relative ability of dietary proteins to stimulate protein regeneration necessitate preliminary depletion of the reserve protein stores. Dogs kept hypoproteinemic by plasmapheresis and low protein diets for long periods may, in this way, be stripped of their reserve material and can produce but little plasma protein. This last fact led Whipple and his group to conclude that fixed tissue protein contributes little, if at all, to the regeneration of plasma protein. However, plasma protein formation has been observed in hypoproteinemic fasting dogs *(24)*, as well as in hypoproteinemic dogs kept on low protein rations *(25)*, it being assumed that the reserve stores were depleted in both cases. Therefore, no conclusive statement as to the capacity of fixed tissue protein to contribute to plasma protein is possible at present.

Synthesis of Hemoglobin

Its function in transporting oxygen to the cells places hemoglobin among the proteins of primary importance to the body. With the emphasis long put on the iron-containing moiety, comparatively little attention has been focused upon the protein, globin, which comprises 95% of the hemoglobin molecule.

Obviously, in addition to iron, materials essential for the synthesis of globin are also limiting factors in hemoglobin production, and Whipple *(26; 27)* has done much to establish the status of the diet in this respect. Using experimental methods similar in principle to those described for the assay of the potency of foods in plasma protein regeneration, he found that whole liver is the most potent foodstuff for hemoglobin formation, with egg white, kidney, stomach and pancreas following in decreasing order. Further work *(28)* revealed that reduced hemoglobin production followed the feeding to anemic animals of diets containing minimal protein but an excess of iron. Confirming these results, Weech, Wollstein and Goettsch *(29)* observed deficient erythrocyte production in 38 dogs subsisting on low protein diets. When the loss of circulating protein resulting from maintenance on the low protein diet for 80 days was considered in terms of the original quantity present, only 30% of the albumin and 50% of the hemoglobin remained in the circulation.

A priori, analysis of the hemoglobin molecule should reveal those amino acids whose administration would yield maximum hemoglobin regeneration. However, none of the 'essential' amino acids found therein (7 'essential' and 8 dispensable amino acids account for 71% of globin *(30)*) seem to be more potent than the others *(31)*. Thus, one gram doses of either glycine, glutamic acid, aspartic acid, cystine, histidine, phenylalanine or proline will increase the hemoglobin output only about 25% above the control level. "Hemoglobin is a peculiar protein and it is possible that its fabrication in the body differs in many respects from that of plasma proteins and tissue proteins" *(32)*. In line with this is Whipple's summary *(33)* concerning possible endogenous sources of hemoglobin: "Hemoglobin in its production may draw on the plasma protein but hemoglobin stands apart in the protein economy and does not con-

tribute freely to the protein pool. On the other hand, the body guards jealously the fabrication of hemoglobin and given a real need for both plasma protein and hemoglobin, the protein flow favors hemoglobin, which under these circumstances always is produced in more abundance than the plasma protein."

Most experiments point to the liver as the organ concerned either with the formation or storage of globin. Biological assays of normal human liver tissue show the equivalent of 120% to 160% of the hemoglobin production factors present in normal animal liver *(34)*. Hypoproteinemia almost always is associated with low reserve stores (60% to 80%) of hemoglobin producing factors.

In spite of the many gaps in our knowledge of the anabolism of hemoglobin, one fact is established, and that is the importance of a high protein intake in promoting maximum hemoglobin synthesis.

Hormone Synthesis

Without taking a position on the question of whether the thyrotropic hormone is the thyroglobulin molecule or thyroxin, nevertheless the constitution of thyroxin provides good presumptive evidence for a relationship between protein (amino acid) intake and hormone synthesis *(35)*. The presence of diiodotyrosine, a derivative of tyrosine, in the thyroid gland lends even further support to this concept *(36)*. Another hormone, that of the adrenal medulla – epinephrine – possesses marked structural similarities to tyrosine and dihydroxy-phenylalanine. In fact, the *in vitro* production of epinephrine from tyramine (the amine of tyrosine) by adrenal medullary tissue has been accomplished *(37)*. Furthermore, the seven identified pituitary hormones are probably protein in nature, and analysis of crystalline insulin has demonstrated

that amino acids comprise at least 88% of its molecule. It is thought, moreover, that the specific properties of insulin may be due to the particular arrangement of the amino acids within the molecule.

Endocrinology is a new science; although enormous advances have been made in the past few years, infinitely much remains to be solved. As some of the complexities of glandular biochemistry become unraveled, it will be most interesting to look for concomitant clarification of many unexplored paths in protein metabolism.

Enzyme Synthesis; Proteins and Vitamins

Within the last decade, the isolation and crystallization of many enzymes have established their protein composition. Some of these, such as pepsin, urease, chymotrypsin, carboxypeptidase, and trypsin, may be simple proteins, while others, *e.g.*, Warburg's respiratory ferment, have been found to be conjugated proteins – proteins associated with a non-protein radical or prosthetic group. According to Northrop *(38)*, the formation of these enzymes is a special case of the more general problem of the synthesis of proteins. Potter *(39)* feels that a considerable fraction of dietary protein finds its way into the multitude of body enzymes, and he states: "One cannot but wonder how much of the protein of the body is enzyme protein and how much is structural protein. At present no answer is available, but there is probably a greater fraction of protein in the form of enzymes than most people would predict, and it seems rather likely that much of the protein of the body which is now considered to have a structural function actually has both a structural and a catalytic function."

The above-mentioned biocatalytic reactions bring up the question of interrelationships between proteins and vitamins,

for it is universally accepted that vitamins have a catalytic function in the body, since they supply neither fuel nor material for structural purposes. "It appears that biocatalysts in general are protein molecules and that when vitamins become part of a biocatalytic system they are elaborated into a special non-protein molecule which is then associated with a specific protein and is called either a coenzyme or a prosthetic group depending on how tightly it is bound to the protein" *(39)*. Since biocatalysts function by effecting the utilization of foodstuffs, complex interrelationships between proteins, vitamins and the enzymes they form are apparent.

Obviously, too, the integrity of many enzyme systems is contingent upon mutually adequate supplies of both vitamins and proteins; probably some of the recent experiments on the effect of vitamins on protein metabolism may be interpreted with this in mind *(40-42)*. Clinically applicable, therefore, is this deduction – successful vitamin therapy depends upon the presence of sufficient amounts of protein with which to form enzyme systems. Recognition of the important role of adequate protein intake in assuring optimum response from vitamin administration is receiving increasing acceptance.

Since the syntheses of proteins are accomplished through enzyme action, protein utilization is explicitly dependent upon previous supplies of protein in the form of enzymes. The synthesis of the first protein supply thus presents a paradox which is answered by the concept of initial spontaneous formation of minute traces of enzyme *(38)*, followed by autocatalytic reactions *(43)* whereby these enzymes are capable of "multiplying" themselves.

Other Non-Protein Nitrogenous Substances

Food protein is of primary importance for stimulating the

production of bile *(44-46)*. The amino acid precursors of bile salts have been shown to be glycine and cystine. Glycine and taurine, a derivative of cystine, conjugate to form glycoholic and taurocholic acids respectively.

The peptide, glutathione, containing glutamic acid in addition to cystine and glycine, is an important tissue constituent intimately involved in oxidation-reduction reactions. Glutathione was discovered in 1921, its constitution established in 1929, and its synthesis accomplished in 1935 *(47-50)*. Besides its oxidative functions, glutathione may play a significant part in the detoxicating mechanisms of the body *(51)*.

Most abundant of the non-protein nitrogenous constituents of vertebrate muscle is creatine, which is liberated from combination with phosphoric acid during muscular contraction, energy being released thereby. Although the origin of creatine was the subject of considerable attention for several years, it was not until 1941 that the use of isotopes definitely proved the precursors to be glycine, arginine and methionine *(52)*. The amount of creatine synthesized daily corresponds to about 2% of the total mass of creatine present in the tissues; this quantity parallels the daily excretion of creatinine, in which form creatine is eliminated *(53)*.

Other physiological substances derived from amino acids are carnosine, a muscle extractive; ergothioneine, found in the blood; and histamine, the amine of histidine. The kidneys and intestines contain a decarboxylating enzyme capable of converting histidine to histamine, thus providing an internal source for this pharmacological pressor substance in addition to its production by bacteria in the intestinal tract, previously mentioned. Reference to histidine also brings up the question of the likelihood of its relation to the purines, important components of nucleic acids *(54; 55)*.

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Section Nine . . . FUNCTIONS OF PLASMA PROTEINS

A Balanced System of Body Proteins

In 1934 Whipple *(1)* first postulated that blood proteins (with perhaps the exception of hemoglobin) take part in a dynamic equilibrium existing between all the proteins of the body, a conclusion recently further substantiated by Schoenheimer *et al. (2)* and Holman *(3)*. This concept carries the implication that food proteins pass through the gastrointestinal tract as amino acids and these amino acids may be combined into aggregates at one point in the body for ultimate use elsewhere *(3)*. These aggregates are transported from the point of synthesis to the point of use by means of the blood stream, during which time they constitute part of the plasma proteins. Much of the synthesis occurs in the liver, although other tissues are probably involved, *e.g.*, antibody (globulin) formation in the reticulo-endothelial system and insulin manufacture in the pancreas (islets of Langerhans). A large part of the equilibrium may be maintained by the blood which can be regarded as an organ several times as big as the liver. A further implication of this concept is that both the capillary endothelium and cell membrane are more or less permeable to these aggregates. (The usual chemical methods do not enable us to differentiate these units from proteins.) Measurements of lymph protein in the body as well as the rapid restoration of plasma protein after acute severe hemorrhage or plasmapheresis are adduced as support to this hypothesis. This viewpoint merely modifies the Starling concept by superimposing a dynamic mechanism.

The state of fluidity among proteins assumes the existence of a reserve store of protein within the organism, mention of which has been made previously in the discussion of plasma protein formation. Under this theory, cell protein is pictured to consist of three parts: a fixed portion, a dispensable reserve

portion, and a transition portion, so-named to indicate its active balance with plasma protein. Definite indications of a reserve store are seen when protein depletion is accomplished by plasmapheresis, but in the production of hypoproteinemia with low protein diets, no such evidence appears *(4; 5)*. Elman and co-workers *(5)* suggest that the concept of reserve protein may not apply to hypoproteinemia produced by dietary means alone.

Osmotic Pressure and Fluid Balance

A second function of plasma proteins relates to fluid exchange between the vascular system and the rest of the body. Two directing forces are normally exerted upon blood circulating within the capillaries, one tending to keep the blood within the vessels, and the other forcing fluid out. If these pressures always counterbalanced each other, nutrient materials would not be carried to nor would waste products be brought from the cells, since both these substances are transported as solutes in fluid media. Apparently those forces causing fluid to leave the capillaries are dominant at the point where nutrient-bearing blood enters the vessels, the arterial end. Contrariwise, the balance of pressure at the venous end is directed toward effecting a return of the fluid, now carrying cellular waste products.

The concentration of albumin and globulin in the blood is one of the most, if not the most, important of the forces tending to keep fluids in or cause their return to the vascular network. This property of plasma proteins arises from their colloid osmotic pressure. Because capillary membranes are relatively impermeable to plasma proteins as contrasted to salt, sugar, non-protein nitrogenous substances and other solutes, the osmotic pressure exerted by the former is the only effective osmotic pressure within the capillaries *(6)*.

Since one gram of albumin exerts approximately three times

as much osmotic pressure as one gram of globulin, the total osmotic pressure (normally about 22 mm. of mercury) is not necessarily proportional to the total protein concentration *(7)*.

It has been demonstrated that the capillary hydrostatic pressure which opposes the osmotic pressure of plasma proteins falls as the blood flows onward through the capillary, thus providing a pressure differential in relation to the relatively constant protein osmotic pressure *(8)*. A schematic illustration *(9)* of the effects of hydrostatic and osmotic pressures upon fluid exchange within capillaries is presented below (Figure 1). The horizontal lines signify capillary walls, with the blood flowing in the direction indicated, while the arrows above the capillary represent capillary blood pressure when they point upward and colloid osmotic pressure when they point downward. Underneath the capillary the diagonal lines indicate the direction of motion of fluid as determined by the difference between the two sets of arrows. It should be realized that forces outside the capillary wall also influence osmotic filtration. Actually, then, each of the vertical arrows illustrates 'effective' pressure; for example, in the case of those arrows representing osmotic pressure, the magnitude given is the difference between the osmotic pressures of the capillary and interstitial fluids. Similarly, the effective hydrostatic pressure is the difference between the capillary blood pressure and the elastic back tension of the tissues.

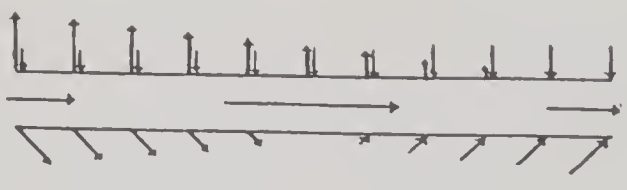

Figure 1

The above discussion on fluid exchange has concerned itself exclusively with the role of proteins. Other factors related to body water balance, the hormonal and salt influences, capillary permeability, etc., must be considered if the subject is to be covered more exhaustively.

Vehicular Function

Plasma protein colloids serve as 'carriers' for many substances in the blood, holding them either by adsorption or chemical combination. Among the products so transported may be mentioned bilirubin, thyroxin, uric acid, cholesterol, carotene and phospholipids *(10)*. About half the serum calcium is combined with blood proteins, its distribution between albumin and globulin possibly following the law of mass action *(11)*. This part of the blood calcium differs from the ionized portion which is regulated by the parathyroids. It is clear, however, that fluctuations in plasma protein levels directly affect total blood calcium concentration *(12)*. The determination of plasma-protein-bound iodine for measuring physiologic thyroid gland activity independently of the structural status of the thyroid gland has been described *(13)*. There is some evidence that other endocrine products are similarly transported *(14)*.

Relations to Immunity

"In the interactions between infectious agent and host and in the complex physiological processes resulting in resistance or immunity to disease the proteins are of the utmost importance" *(15)*. This conclusion, emphasized by innumerable experiments in immunochemistry from the beginnings of this science to date, refers not only to the chemical composition of the parasitic invaders but also to that of the body defenses, since antibodies have been found to be globulin molecules whose synthesis is somewhat altered when antigen enters

the body *(16)*. With globulin in part, at least, originating in reticulo-endothelial cells, antigen can be defined as "a substance which can specifically modify the synthesis of the cytoplasm of the cells of the reticulo-endothelial system" *(17)*. Since most antigens are proteins, both members of the antigen-antibody mechanism must be considered from the viewpoints of modern protein chemistry. This discussion is concerned only with plasma protein however; present consideration, therefore, is confined to antibodies.

If antibodies are modified globulins, "it follows that antibody-production must be influenced by the same conditions which determine globulin-production" *(18)*. Whether this is basically referable to an adequate diet of proper amino acid content has not been conclusively determined, though most experimenters come to such a conclusion despite the definitely contrary observations mentioned previously. (See p. 30.)

The recent report by Schoenheimer *et al. (19)* that antibody participates in metabolic reactions involving dynamic exchange with dietary nitrogen strongly suggests a positive correlation between food intake and antibody production. The importance of a luxus protein diet in providing the basic materials for antibody formation has been stressed from reasoning that the immunologic potential of the reticulo-endothelial system varies quantitatively with the locally available protein reserve.

Other Functions

A very small proportion of the alkali-binding power of the blood is attributable to plasma proteins. Their effect in regulating the acid-base balance is of such small magnitude that even marked hypoproteinemia scarcely impairs the buffer system. Also, the roles of fibrinogen and prothrombin in blood clotting must be mentioned here, since a strict interpretation of the

term plasma proteins necessarily includes these components. (See p. 28.)

A summary of the functions of plasma proteins cannot but lead to the conclusion that many former ideas concerning their static nature are no longer tenable. Rather than behaving as inert colloids, plasma proteins participate extensively in facilitating fluid, nutrient, and protein exchange, and also function as important links in the body's defense against bacterial invasion through immune response. In plasma proteins, then, we indeed possess a dynamic blood constituent.

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Section Ten . . . DETOXIFYING ACTION OF PROTEINS

The process by which foreign chemicals are rendered innocuous to the body is known as detoxication. Included among the reactions of detoxication are those involving conjugation with amino acids and related compounds. As simple examples, benzoic acid is converted to hippuric acid by conjugation with glycine, the effects of phenylacetic acid are minimized by the action of glutamine, the amide of glutamic acid, and bromobenzene is eliminated as a mercapturic acid by combination with cysteine *(1)*. Not all detoxication reactions are so easily understood and explained, however. In most cases, the protective effects of a high protein intake against certain pharmacologic agents have not yet been traced to specific amino acid constituents.

The liver seems to be principally affected by the toxic nature of many foreign substances. Up until the last few years it had been thought that a high glycogen content gave the liver maximal protection against foreign chemicals but many reports indicate that carbohydrate acts secondarily through its protein-sparing action *(2-4)*. A high protein rather than a high carbohydrate content provides the best defense for the liver against a variety of damaging agents: *e.g.*, chloroform, arsphenamine, dimethylaminoazobenzene, and naturally occurring wheat selenium *(5-8)*. Increased tolerance to sulfa drugs also may be brought about by high protein diets *(9)*. The clinical implication of resistance to toxins as conveyed by adequate protein intake will be considered later. (See p. 69.)

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To round out this brief survey of normal protein metabolism, values for the actual range of normal concentrations of nitrogenous constituents of the body are presented in Table V.

Perhaps the most valuable means for evaluating the protein-nutritive state of an individual is the determination of the plasma protein level. Actually, such estimation should be done routinely in any condition where protein metabolism is likely to be affected – dietary insufficiencies, liver disorders, surgical procedures, burns, kidney disorders, shock, etc.

Many methods exist for the quantitative determination of plasma proteins. Most reliable, of course, are those technics based on actual determination of protein nitrogen on a sample of plasma (or serum). In consequence of the discovery that a parallelism exists between density and plasma protein content, simplified procedures for finding the level of these proteins in the blood have been devised *(1-8)*. These technics include density determination, measurements of the time required for a sample drop of known volume to fall between marks in a medium of known density, or, even more crudely, observations of whether or not a drop of sample is lighter than, equal to, or heavier than one of two or three test mixtures of known densities. The specific gravity of normal plasma is reported to be 1.0264 ± 0.0018, corresponding to 6.64 ± 0.61 gm. of plasma protein per 100 cc.

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TABLE V — *Physiologic Normals of Nitrogen Metabolism*

CONSTITUENT	BLOOD (PER 100 CC.)	URINE (24 HOUR SAMPLE)
Plasma Proteins	6.0–7.2 gm.	
Albumin	4.6 ± gm.	
Globulin	2.0 ± gm.	
Fibrinogen (plasma)	0.3–0.6 gm.	
Non-protein Nitrogen	25–35 mg.	
Urea	20–30 mg.	25–35 gm.
Urea Nitrogen	10–15 mg.	11.7–16.3 gm.
Uric Acid	2–3.5 mg.	0.6–1 gm.
Ammonia Nitrogen	0.1–0.2 mg.	0.5–1 gm.
Undetermined Nitrogen	4–18 mg.	0.7 gm.
Amino Acid Nitrogen	4–4.5 mg.	
Free Amino Nitrogen	5–8 mg.	0.2–0.4 gm.
Creatinine	1–2 mg.	1.2–1.7 gm.
Creatine	3–7 mg.	
Inorganic Sulfur	0.1–1.1 mg.	1.7–2.7 gm.
Ethereal Sulfur	0.1–1.0 mg.	0.15–0.3 gm.
Neutral Sulfur	2.2–4.5 mg.	0.2–0.4 gm.
Indican		0.005–0.01 gm.
Hemoglobin	14.3–17.4 gm. ♂ 12.8–15.7 gm. ♀	
Hematocrit	43.5–50.3 ♂ 38.0–46.3 ♀	

Albumin/Globulin ratio—1.38–2.2.

PART TWO

ALTERED

PROTEIN METABOLISM

The preceding section dealt with the normal metabolic processes to which ingested protein is subjected in its course through the body. Here, it is intended to consider the physiological aspects of altered protein metabolism and the causes of the protein depletion most commonly encountered.

It will be seen that the causes of protein depletion are multiple and its consequences rarely, if ever, singular or uncomplicated. Study of the preceding sections conveys so strong a feeling of the physiologic ubiquity of amino acids that profound systemic disturbances are to be anticipated not only as a result of their withdrawal but even as a result of minor irregularities in their metabolism.

Despite its specific meaning – low blood proteins – the term hypoproteinemia is also employed in a broad sense to indicate a general depletion of body protein. Because it is more difficult to measure losses of tissue or organ protein, and because it has been shown that decreased plasma protein is accompanied by tissue protein depletion (in fact, 30 times as much nitrogen is lost from body tissues as from serum protein during protein depletion *(1)*), plasma protein levels are used as an index of the protein nutrition of an individual. Hence, in discussing protein deficiency, the term, hypoproteinemia, may be used in a somewhat synonymous way.

The term 'somewhat synonymous' as used above, was written to imply the difficulties involved in interpreting plasma protein figures in relation to the extent of protein depletion. One of the reasons for this lies in the fact that protein concentration may be altered not only by changes in total protein content but also by changes in blood volume. The normal ratio of the volume of packed red cells to total blood volume (hematocrit) is about 0.45. In general, a fall in hematocrit indicates plasma volume increase. Low hematocrit, therefore, reveals blood dilution. Values above 45% mean that blood volume is decreased (dehydration) and plasma protein concentration is higher than at normal blood volume. The actual total protein in the circulation is thus lower than the concentration suggests. In other words, hypoproteinemia may not be revealed by the plasma protein level when dehydration of the blood coexists. Clearly, hematocrit readings are required whenever uncertainty about the maintenance of blood volume casts doubt on the figures for plasma protein concentration.

Far better than hematocrit readings are measurements of total blood volume; from this, total plasma proteins can be calculated. In those cases where simultaneous reduction of red blood cells and plasma volume occurs, the ratio of one to the other is undisturbed, and hematocrit readings appear normal. Under these circumstances, a normal plasma protein content registers as hyperproteinemia; true hypoproteinemia is masked by apparently normal values. Only circulating

blood volume measurements can reveal the concomitant occurrence of extreme dehydration, anemia and hypoproteinemia.

Additional valuable diagnostic aid may often be obtained by separate determinations of the albumin and globulin levels. This is especially indicated when the total plasma protein content and the hematocrit value are normal, yet protein deficiency is strongly suspected from the overall clinical picture. Since albumin and globulin levels fluctuate independently of each other in response to different influences, their respective concentrations may yield important clues as to the patient's nitrogen status. When protein intake is inadequate, albumin mainly is reduced: reduction in total plasma proteins under such circumstances is due to reduction in albumin. In severe infectious conditions, globulin is increased. Those cases of malnutrition having concomitant infection may therefore have total plasma protein values within the normal range of concentration, yet actually composed of an excessive amount of globulin and reduced albumin.

More or less marked edema may occur in patients having total circulating plasma proteins within the normal range as described above. The apparent paradox is explained as follows: the osmotic pressure exerted by proteins contributes to maintenance of fluid balance. Albumin has greater osmotic pressure than globulin. The low osmotic pressure which is exerted by a normal total plasma protein composed of excess globulin and reduced albumin permits edema to develop. Investigation of the osmotic pressure would seem to be a logical procedure in such cases. However, it is a simpler technical problem to determine albumin and globulin concentrations than to determine osmotic pressure, and charts are available from which osmotic pressure can be calculated from albumin and globulin concentrations *(2)*.

More and more, the albumin-globulin ratio is being regarded as possessing little significance in diagnosis. Reduction of the A/G ratio may arise from changes of one or both components, either from a reduced albumin or an increased globulin. Since the A/G ratio does not, of itself, indicate which of the two has changed, it is far more valuable to know the actual concentrations of albumin and globulin than their ratio to each other.

Another factor bearing upon plasma protein levels is the state of the protein reserves. Plasma protein levels can be maintained at the

expense of reserve stores. Except in acute cases, reduction of blood proteins does not occur until these stores are depleted. An individual may, therefore, be approaching the hypoproteinemic state as his reserves become used up, yet examination of the blood proteins will show normal values. If this individual unexpectedly requires large amounts of proteins, or if his intake be drastically reduced as in the postoperative period, acute edema may suddenly develop.

While it is not possible to assay the reserve stores with any degree of accuracy, clues as to the likelihood of their being depleted can often be deduced from the patient's dietary and weight record and from any of the several liver function tests which have been described in the literature *(3; 4; cf. 5)*.

When interpreting blood protein values, the physician also must consider the possible existence of an abnormal permeability to proteins. Blood protein levels cannot be an index of body nutrition if the walls of the vascular system are abnormally permeable to them. Disturbances of the normal nitrogen exchange between the body cells and the blood result from alterations of the vascular endothelium. Such alterations are especially apt to occur in shock, anoxia and burns. When the question of altered capillary permeability arises, determination of the protein content of interstitial fluid – that is, edema fluid – is of value.

The causes of decreased protein levels in the animal organism are traceable to the basic metabolic pathway beginning with intake, proceeding to utilization, and ending with excretion. Thus, protein deficiency states may be considered as due either to insufficient intake, to faulty utilization or to excessive loss. By some investigators *(6)* who have regarded the liver as the focal point of protein regeneration, protein deficiencies may be classified as pre-hepatic, hepatic or post-hepatic. Deficiencies of the first type – pre-hepatic – arise when the supply of amino acids offered to the liver is inadequate because of poor intake, defective digestion or absorption, or bacterial action in the intestinal tract. Hepatic causes of protein depletion result from impaired liver function primarily with respect to plasma

protein fabrication. The post-hepatic type is comparable to the excessive loss group previously mentioned, except that the location of the loss is specifically defined. Although the classification used below is not based upon these distinctions – pre-hepatic, hepatic, and post-hepatic – nevertheless this method of visualizing causation is somewhat helpful to an 'over-all' view of protein deficiencies.

Inadequate Intake

Insufficient intake of protein-rich foods of high biological value is responsible for most cases of hypoproteinemia. That hypoproteinemia actually results from decreased protein ingestion has already been shown in the section concerned with the formation of plasma proteins. (See p. 30.) However, since those experiments dealt specifically with the synthesis of these proteins rather than their concentration levels, it might not be repetitious to mention a few of the many investigations more directly pointed toward the latter factor. For example, in a study of 24 undernourished Chinese *(7)*, their serum protein concentration was found to range from 2.7% to 4.93%, values significantly below the normal. A year previously, low protein levels were demonstrated in 57 hospital patients showing evidence of malnutrition *(8)*. Since reduction of serum proteins has been experimentally produced in white rats fed a protein deficient diet *(9)*, the relation of cause and effect between inadequate protein intake and hypoproteinemia has been established both clinically and in the laboratory. It is noteworthy, however, that in some cases of protein malnourishment, dehydration and hemoconcentration may mask hypoproteinemia, so that it is not apparent without restoration of blood volume *(10)*.

During periods of famine, hypoproteinemia and its most obvious visible manifestation, edema, have often been observed *(11; 12)*. Lusk *(13)* has calculated that the average pro-

tein intake in Germany during 1916 and 1917 was 31.1 gm. a day, an amount definitely below even minimum maintenance levels. Here, of course, the protein malnutrition was a primary condition caused by actual lack of protein foods.

When the caloric intake itself is low, an otherwise adequate amount of protein is disproportionately used for energy. In order to get maximum nitrogenous benefits from protein intake, it is essential that an ample supply of protein-sparing carbohydrate be simultaneously ingested. Lack of effective rather than actual amounts of protein may thus cause hypoproteinemia.

Other causes of protein malnutrition are referable to an individual's taste, appetite or whim. Thus, hypoproteinemia may result from self-imposed vegetarian regimes or stylish reducing diets. In the aged, lack of appetite often cuts protein intake far below minimum requirements. As Tuohy *(14)* states, "Hunger is a natural instinct with the young; it is often a luxury with the old". Alcoholic addicts, likewise, consume too little protein as well as other dietary essentials, while the clinical syndrome, anorexia nervosa, presents a picture of extreme aversion to food of any kind *(15-17)*. The lowered blood proteins found by Gettler and Lindeman *(18)* in 76% of their cases of pernicious anemia are to be explained, in part, by concomitant anorexia, although it is not to be denied that other factors such as impaired digestion also participated.

Protein malnutrition is often the consequence of various pathological conditions. Patients may purposely reduce their food intake because of pain or discomfort; they may be overzealous in carrying out prescribed diets; or, they may be physically unable to eat. Some clinical conditions from which these situations can arise are stricture of the esophagus, indigestion, gastric or duodenal ulcers, carcinoma of the esophagus or stomach, and rectal stricture *(6)*.

Elimination diets in allergic states may contain insufficient protein, especially when the suspected or known allergens are protein foods such as milk, eggs, or cereals; allergies to these foods are particularly common in children, whose need for protein is relatively great. Similarly, diabetic patients who eat poorly balanced diets over periods of time may develop hypoproteinemia when their limited protein reserves become exhausted *(19)*. An otherwise adequate protein intake may not cover the heightened requirements in thyrotoxicosis, fevers and infections. Payne and Peters *(20)* have shown that hypoproteinemia frequently develops as a result of malnutrition in people with heart disease.

Faulty Digestion and Absorption

If the quantity of digestive juices and enzymes secreted into the gastrointestinal tract is substantially diminished, the breakdown of proteins into amino acids cannot be completed, absorption likewise suffers, and hypoproteinemia ensues. Such a situation is observed in achylia gastrica, achylia pancreatica, and in achlorhydria associated with pernicious anemia, gastrectomy, gastritis or other causes *(21-25)*. The indiscriminate use of antacids or bismuth preparations for dyspepsia or other digestive disturbances may cause further impairment of digestion by inhibiting as well as by adsorbing digestive secretions. Even in the initial stages of treating inanition by administering highly nutritious diets, difficulties may be experienced in that the food taken in may not be digested on account of concomitant atrophy of the alimentary tract glands *(26)*.

Defective absorption of protein split products may occur in connection with jejunostomy, ileostomy, ileocecal or jejunocolic fistula, regional enteritis, tuberculosis of the small intestine, and malignancies of the intestinal tract *(27-29)*. Kantor *(30)* has applied the term sprue syndrome to designate all dis-

cal or chemical agents are other causes of heightened capillary permeability *(42; 43)*. Clinically, these situations are observed in association with inflammatory processes such as abscesses or tuberculous pleurisy *(40, p. 697)*, interference with the circulation by intestinal obstruction or distention *(44; 45)* and prolonged decline in blood volume and pressure subsequent to hemorrhage *(46)*. Since one of the manifestations of the shock syndrome is an increased permeability of the vascular endothelium *(47)*, the occurrence of hypoproteinemia may be anticipated whenever shock develops.

The importance of fluid transudates as causes of protein loss is emphasized by the fact that the serous fluid accumulating after mechanical or thermal trauma contains as much protein as blood plasma itself *(48)*. Human ascitic fluid obtained from patients with portal cirrhosis of the liver or cardiac decompensation has a protein content equivalent to about 28% to 45% of blood plasma, a circumstance which has led to its successful experimental use in combating hypoproteinemia and shock *(49)*.

Pus obtained from abscesses is reported to consist of as much as 8.1% protein, of which almost three-fifths is albumin *(50)*. In patients having chronic purulent drainage as in peritonitis, osteomyelitis, etc., pus formation can be a significant contributory factor in bringing about hypoproteinemia *(40, p. 697)*.

Trauma, especially that due to prolonged surgical procedures, often gives rise to large protein losses. Trauma increases both endothelial permeability and tissue protein breakdown. A further cause of protein loss is the hemorrhage incidental to trauma.

Increased Metabolism

Reduction of circulating and tissue proteins may occur dur-

ing periods of accelerated protein metabolism when the protein intake is not proportionately increased. Augmented protein metabolism may be physiologic, as in pregnancy and lactation, or pathologic, as in fevers and thyrotoxicosis. During pregnancy the growth of the fetus presents a nitrogen demand which must be satisfied by the tissues of the mother if not by the food. Postpartum, the proteins of milk are formed in the mammary gland from amino acids circulating in the blood stream *(51)*, and since 13 to 18 grams of milk protein are secreted daily by the average lactating woman *(52)*, it is evident that either the mother or the milk supply will suffer if the protein intake is not increased accordingly.

Of the pathological conditions mentioned, although the rise of the basal metabolic rate to plus 30 and even plus 70 in hyperthyroidism represents an extremely accelerated rate of tissue catabolism, the average 7.2% increase in the basal metabolic rate for each degree Fahrenheit rise in temperature during fevers is not to be regarded as insignificant with respect to protein needs and losses *(53)*. In fact, a marked drop in the serum proteins of patients given malarial fever therapy was recently noted *(54)*.

Impairment of Protein-Regenerating Mechanism

A number of years ago, several cases were reported in the literature *(55; 56)*, the hypoproteinemia of which seemed to be due neither to excessive loss nor inadequate intake of protein, but rather to an impairment of plasma protein production. Postmortem examination of one of these seemingly idiopathic cases of hypoproteinemia revealed atrophy of the intermediate and peripheral liver lobule cells, suggesting defective function of this organ as the cause of the low protein levels *(56)*. In agreement with this is the fact that in clinically recognized liver disorders – portal cirrhosis, syphilis of the liver, tuber-

culosis of the liver, subacute yellow atrophy – hypoproteinemia and edema are usually observed *(57-59)*. There is evidence, also, of impairment of plasma protein regeneration in the nephrotic syndrome *(60; 61)*. Decreased liver function in hyperthyroidism may also be considered as an etiological factor in the hypoproteinemia often present in this metabolic disorder *(59; 62-64)*.

Infections also appear to have an inhibiting influence upon the body mechanism for producing plasma proteins. Laboratory evidence of this has been furnished by Whipple's group *(65; 66)*. Experimentally produced turpentine abscesses were found to disturb plasma protein synthesis in their plasmapherized dogs. Impairment of plasma protein regeneration in patients suffering from infection, with or without suppuration, has been noted by several investigators *(e.g., 6)*.

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Most cases of protein depletion result from multiple rather than individual factors. A patient with chronic osteomyelitis may become hypoproteinemic because of a combination of inadequate protein intake, loss of protein in pus, and accelerated protein metabolism due to fever. Anorexia in conjunction with poor digestibility often precipitates a hypoproteinemic state in patients with gastric disorders. Prolonged vomiting in a pregnant woman produces a relatively more severe protein depletion because of a greater need. Generally, it may be stated that inadequate protein intake, or at least a relatively inadequate protein intake, is the predominant factor in the majority of patients exhibiting hypoproteinemia.

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62 . . .

Section Two . . . PHYSIOLOGICAL AND PATHOLOGICAL EFFECTS OF PROTEIN DEPLETION

Disturbances of Water Balance

One of the most striking consequences of hypoproteinemia is that related to its role in regulating the water balance of the body. It will be recalled that maintenance of blood volume is due to a balance between those forces tending to cause fluids to leave the capillaries and those tending to cause the return of interstitial fluid to the vessels. (See p. 40.) The colloid osmotic pressure exerted by plasma proteins is the principal intravascular force drawing fluids into, or checking their escape from, the capillaries. When colloid osmotic pressure is reduced by hypoproteinemia, blood volume is decreased because the forces causing fluid to filter out of the vessels are relatively greater than those causing it to return. The situation may be presented graphically (Figure 2) in the same manner as the normal fluid balance was depicted on page 41.

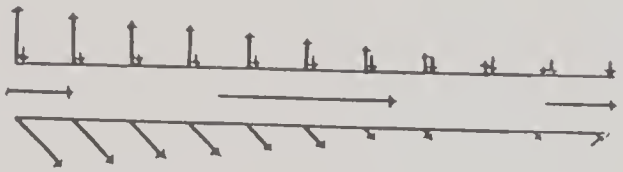

Figure 2

The ultimate results of reduced colloid osmotic pressure must be considered separately with respect to both sides of the capillary membrane, that is, reduced blood volume and increased interstitial fluid volume, each of which results in further physiological disturbances.

Reduced Blood Volume: Shock, regardless of the cause, is essentially peripheral circulatory failure resulting from a discrepancy between the size of the vascular bed and the volume

of intravascular fluid *(1)*. When a moderate reduction of blood volume occurs, the peripheral capillaries contract, thereby compensating for the difference between the volume capacity and the actual volume. In severe hypoproteinemia, however, capillary absorption may, on account of diminished osmotic pressure, be so reduced that the ensuing extreme reduction in blood volume can only be partially compensated. Under such conditions, the volume output of the heart is reduced, blood pressure begins to fall, and anoxia results. Since oxygen lack increases capillary permeability as much as fourfold *(2, p. 45)*, the small amount of protein present in the blood stream will be reduced even further by outward diffusion. This brings the situation right back to where it started – to hypoproteinemia and reduced blood volume – except that now the blood volume is further depleted because of an even lower colloid osmotic pressure. Stated more succinctly, circulatory deficiency causes anoxia, and anoxia causes circulatory deficiency. Moon *(2, p. 209)* calls this the 'cycle of death' in shock.

Although experimental shock from hypoprotcinemia in plasmapherizcd dogs has been reported *(3)*, no reports of similar cases of shock due solely to hypoprotcinemia in humans have appeared. Undoubtedly, however, the presence of hypoproteinemia predisposes toward greater vulnerability to shock initiated by other factors. Of course, shock superimposed upon pre-existing hypoproteinemia presents a morc difficult therapeutic problem than shock in the presence of ample protein reserves.

Increased Interstitial Fluid Volume: The effect of hypoproteinemia upon interstitial fluid volume has received much more attention than the concomitant intravascular manifestations. Perhaps this is a reflection not only of a morc easily observed phenomenon but also of a situation less subject to

physiologic compensation. Thus, although increases in interstitial fluid volume may not be immediately, or at least strikingly, apparent, a point is soon reached at which clinical edema manifests itself – to the great distress of the individual so afflicted.

The causal relation between low plasma protein levels and the occurrence of edema was not recognized until after World War I. Kohman *(4)*, impressed by the picture of dropsical, starving Europeans, found that she could reproduce a similar edema in rats by feeding them diets composed largely of carrots; from this work she was led to conclude that the edema resulted from insufficient intake of proteins. In 1929 measurements of plasma protein concentration in experimental edema were carried out. At that time, not only was confirmation of these experiments obtained, but also it was shown that the reduced protein intake was followed by reduction of plasma proteins, which, in turn, gave rise to edema. Other experimenters *(5-8)*, utilizing plasmapheresis as well as low protein diets, have definitely established that hypoproteinemia will cause edema.

The severity of edema parallels the extent of plasma protein depletion, all other factors being equal. Assuming a 'critical level' of plasma proteins to be that concentration below which edema will appear, experimental and clinical work has determined this to occur at about 5.5 gm. of total protein per 100 cc. of serum or 2.5 gm. of albumin per 100 cc. *(9; 10)*. The use of a 'critical level' for albumin is based upon Govaerts' work *(11)* showing that albumin possesses nearly 4 times as much osmotic pressure as globulin. Hence, in clinical conditions where albumin is decreased and globulin increased (due to infections evoking immune processes), the total serum proteins may be normal, yet the colloid osmotic pressure would be low and edema would impend.

More recent work *(12-14)* suggests that Govaerts' ratio may be incorrect, and that, gram for gram, the osmotic pressure of albumin may be either two and one-half times that of globulin, or perhaps equal to it.

Undoubtedly a more accurate way of determining a critical level for edema is to define it in terms of colloid osmotic pressure; if so, the fulcrum is at about 15 mm. of mercury, the normal colloid osmotic pressure being about 22 mm. of mercury *(15; 12; 16)*. Osmotic pressure may be calculated from clinical laboratory data (see p. 51).

The above statements concerning the role of plasma proteins in the phenomenon of edema do not imply that other factors do not operate or are of no significance. On the contrary, several of them have considerable influence in regulating water balance. Obviously, if the fluid intake is reduced, the tendency to edema at a particular plasma protein level will be lowered. Salt intake, too, often is the decisive factor in regulating the appearance or disappearance of edema, especially if the protein concentration is at or about the critical level *(10; 17-19)*.

Continued edema despite the administration of large quantities of protein may have its origin in abnormal capillary permeability to proteins. Increased blood pressure is another item to be considered; in the edema of heart disease, it assumes primary importance. Since protein malnutrition often occurs in heart disease *(20)*, the lowered colloid osmotic pressure would accentuate the tendency to edema as shown in Figure 3. (cf. Figure 1, p. 41.)

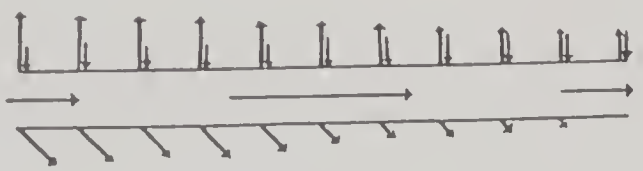

Figure 3

Retardation of Wound Healing

The course of wound healing is divisible into several distinct periods, the first of which is the latent stage. During this time, extending from 1 to 4 or 5 days, the wound remains practically the same size. Subsequent periods involve the processes of contraction, epidermization, and cicatrization. The velocity of all four stages of wound healing is determined by a considerable number of variables, many of which – for example, type of suture material, technique of surgical incision, and nutritional intake – are controllable by the physician.

One of the first observations concerning the effects of diet on wound healing was that made by Clark in 1919 *(21)*. The six day latent period observed in his fat-fed dogs was eliminated when he gave the animals a high protein (meat) diet. The period of contraction began at once, so that the entire healing period was shortened in proportion to the reduction in the quiescent period. On the other hand, it has been reported *(22)* that high protein diets shortened not the latent period but the stage of connective tissue proliferation, that is, the period when fibroblasts multiply into a cicatrix. As a result, the maximum strength of the wounds was attained much sooner than with diets containing only moderate amounts of protein. Despite these differences as to the effects of protein on the various stages of wound healing, there is no longer any doubt that proteins accelerate and stimulate cellular proliferation in both injured and normal tissues *(23)*.

Wound disruption is a complication encountered in 1% to 3% of all abdominal operations *(24; 25)*. Experimental work in 1938 *(26)* on opened wounds in dogs revealed that 72% of the animals had an associated hypoproteinemia; when plasma transfusion given immediately after operation corrected the hypoproteinemia, the delay in wound healing was averted.

First to report serum protein studies on humans in connection with wound healing were Koster and Shapiro *(27)*. From their investigations they concluded that "the poor nutritional state of which hypoproteinemia is a manifestation may favor both the development of deep infection and disruption of clean wounds". Of particular interest is the observation that 68% of those patients with disrupted wounds were operated upon for lesions of the duodenum or stomach, or for cancer of the gastrointestinal tract *(28)* – all conditions in which hypoproteinemia is common as a sequel to inadequate intake and poor digestion of proteins. In a series of twenty cases of wound disruption showing hypoproteinemia and hypovitaminosis C, the average serum protein concentration was 5.83 gm. per 100 cc. "It would seem logical that, if normal healing is to be expected, the ascorbic acid and serum protein levels of the blood plasma ought to be brought to and kept at normal concentration" *(25)*.

Often, in the insistence upon a sufficient supply of calcium for bone growth and repair, the protein needs of the bones are overlooked. Hypoproteinemia may restrict bony proliferation in three ways: [1] Since it has been shown that a large fraction of serum calcium is bound to serum proteins, reduction in plasma proteins results in concomitant reduction of blood calcium. (See p. 42.) [2] Very recent studies *(29)* have disclosed, also, that calcium absorption in the intestines increases directly as the protein intake is increased. It is the opinion of the investigators that, were no protein or amino acids ingested, scarcely any calcium would be absorbed. [3] In fracture healing, fibroplasia takes place just as in wound healing. Artificially produced fractures in hypoproteinemic dogs have been followed by slow formation of bony callus *(30)*. Rhoads and Kasinkas *(30)*, in discussing the various mechanisms by which the callus retardation in their hypoproteinemic dogs might be explained, also mention that reduction in plasma volume and the impair-

ment of circulation resulting therefrom may be a contributing factor. Regardless of our inability to explain precisely how the hypoproteinemic state adversely affects callus formation, one cannot but conclude that fracture cases require high protein as well as high calcium intakes.

Lowered Resistance to Infection

A rather serious conscquence of protein depletion is that having to do with lessened resistance to infection. Dogs stripped of their reserve as well as plasma proteins are not as resistant as control animals. In fact, they are even more susceptible to infection than anemic dogs *(31)*. Thus, they often succumb to abscesses, respiratory infections, septicemia, endocarditis and enteritis *(32)*. Correspondingly in man, pneumonia and pulmonary edema often have terminated extreme cases of hypoproteinemia *(33)*. The decreased resistance may be due to depletion of the essential protein matrix of thc body cells, to impaired antibody (globulin) production, to altered water balance, or to a combination of all of these factors. Since, however, the precise mechanism of resistance is unknown, knowledge of the exact manner in which proteins influence resistance awaits further research. Meanwhile, available facts are of sufficient magnitude to strongly verify that optimum protein intake is of considerable importance in determining and maintaining cellular resistance.

In human beings, simple clear-cut protein deficiencies are rarely, if ever, observed. Other deficiencies simultaneously occurring may accentuate the decrease in cellular stamina caused by protein depletion so that only after consideration of these as well as the protein factor will resistance approach a maximum.

Liver Function and Resistance to Intoxication

Another manifestation of hypoproteinemia, one rather closely

allied to the above-mentioned infectious state, concerns decreased resistance to intoxication. Here, the protein influence seems to be mediated more through the liver than through the body cells generally. In view of the adverse effects of many chemicals, particularly anesthetics, upon liver function, the experiments of Miller, Ross and Whipple *(34)* are most revealing. Well-fed dogs were found able to withstand an hour's administration of chloroform without evidence of intoxication, whereas fifteen to twenty minutes of the same anesthetic to plasma-depleted dogs caused liver necrosis and death. Similarly, diminished liver resistance to arsenicals has been observed in these animals *(35)*. Large protein feedings promptly restored the normal threshold of susceptibility to these dogs.

Especially noteworthy are those reports concerning specific amino acids in relation to liver susceptibility. It appears that methionine is the key amino acid for increasing the resistance of this organ in protein depleted dogs *(36)*. If given shortly before anesthesia, methionine will provide complete protection. It will even impart significant resistance to the liver when given as late as three to four hours after chloroform. The effectiveness of this amino acid may possibly be related to its sulfur content since the livers of protein depleted dogs have been shown to be proportionately more deficient in sulfur than in nitrogen. However, other work suggests that the methyl group (CH_3) is also an important part of the methionine molecule.

"The effects of protein depletion on the liver are of general interest not only because of the increased susceptibility to obvious hepatotoxic agents, such as chloroform or arsphenamine, but also because of its significant relationship to other disease states – fatty livers, experimental cirrhosis, experimental liver carcinoma, and decreased liver function" *(36)*. Histologically, low protein diets have resulted in diminution of stainable liver cytoplasm and atrophy of the lobule cells *(37; 38)*. Chemically,

a definite fall in liver protein content ensues *(37; 39)*. Functionally, liver impairment does result, although some liver tests may not reveal the insufficiency, either because they are not sensitive enough or because they test unrelated or unaffected liver functions. Thus, normal galactose tolerances but reduced iso-iodeikon and Rose-Bengal excretion are observed in hypoproteinemic dogs *(37; 40)*. In humans, tests with bromsulfalein and dextrose also are normal, yet Quick's hippuric acid test usually reveals marked functional impairment *(41)*. Apparently, therefore, the latter test is a more sensitive indicator of this type of liver function in the human. All the hypoproteinemic patients of a recently reported series *(42)* showed subnormal liver function by the hippuric acid test.

Hypoproteinemia may result in subnormal liver function with respect to two phases of protein metabolism, deamination and plasma protein synthesis. In contrast to the normal individual, amino acids injected into hypoproteinemic individuals are retained in the blood stream *(43; 44)*. This is considered evidence of impairment of liver function, at least with respect to deamination. As regards plasma protein formation, Youmans *(45)* has observed that restoration of serum proteins to normal levels in protein depleted patients is much slower in chronic than in acute deficiencies. Similar disturbances of plasma protein regeneration in dogs subjected to prolonged depletion of body protein have been noted *(46)*. Here then is a vicious cycle – hypoproteinemia leading to decreased liver function, and decreased liver function causing further hypoproteinemia. It is fortunate however, that this dangerous situation arises only when severe protein deficiencies are indefinitely prolonged.

Other Effects of Protein Depletion

Hypoproteinemia does not adversely influence cardiac function as determined by the electrocardiograph *(42)*. The effect

of protein depletion upon renal function is not clear. Leiter (47) has produced nephrotic albuminuria in dogs subjected to plasmapheresis. In human beings afflicted with nutritional edema, the development of either polyuria or oliguria may depend upon the water content of the diet; nocturia, unexplainable on this basis, occasionally occurs. Postmortem studies of nutritional edema have yielded conflicting evidence with regard to kidney weights *(48, p. 384)*. The kidneys of albino rats, however, have been reported to lose from 18% to 28% of their original protein content after a seven day fast *(49)*. Whether such losses of organ protein do occur in starving humans and if so, how they affect renal function are not known. It seems likely, though, that in severe cases of protein depletion, with reduction of blood volume and loss of protein from the kidney, altered renal function might result.

Muscular atrophy and emaciation always result from prolonged protein restriction. Ever-present symptoms of protein deficiency, therefore, would be abnormal fatigue, a dislike for strenuous activities, and general lassitude. Often the possible role of protein deficiencies in connection with the fatigue syndrome is overlooked clinically.

In prolonged states of depletion, the production of digestive enzymes, hormones, bile acids and hemoglobin, doubtless, is also limited. The frequency of achlorhydria and of lowered basal metabolism in nutritional edema is consistent with this statement. Diminution in size and secretory activity of the thyroid gland was particularly emphasized by those who studied nutritional edema in Europe in 1918 *(48, p. 442)*. Anemia was also almost constantly observed *(48, p. 254)*. Even the lowered birth rate in Germany during World War I was linked to protein lack *(48, p. 254)*; this might be correlated with Holt's observations *(50)* on the effects of arginine deficiency in depressing human spermatogenesis.

Limited growth is to be expected as a consequence of prolonged protein inadequacy in view of the fact that the main organic constituent of cellular protoplasm is protein. Although simple protein deficiencies have never been reported in humans, studies during the last war on Belgian and German children who were subsisting on low protein diets revealed a general retardation of 1 to 4 or 5 years in height and weight *(48, p. 84)*. Weight figures may be misleading when water retention is present, but subnormal height certainly is objective enough. It is fortunate that proper feeding of such malnourished children usually results in rapid return to normal.

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Section Three . . . OTHER ABNORMALITIES OF PROTEIN METABOLISM

Inborn Errors of Metabolism

Inborn errors of metabolism are a group of metabolic disorders in which a normal biochemical reaction is either not carried out or is not completed. No special pathology is observed and the individuals so afflicted may have a normal life span; the main, and more often the only, sign of the disorder is an increased excretion of an abnormal urinary constituent, usually an intermediate product in the metabolism of a particular body substance. Those inborn errors related to protein metabolism are cystinuria, alkaptonuria, phenylketonuria and tyrosinosis *(1)*.

Failure to oxidize the amino acid, cystine, results in cystinuria. Individuals who have this disorder are particularly prone to develop renal calculi because of the low urinary solubility of cystine. In alkaptonuria, the urine becomes oxidized on contact with the air and gradually turns black. This is due to the presence of homogentisic acid, an abnormal metabolite of both phenylalanine and cystine. Phenylketonuria seems to occur only in mental defectives, and is apparently due to an inability to dispose of phenylalanine. Tyrosinosis is a rare clinical entity, there having been only one reported case in the literature *(2)*. It appears to represent an inability to carry the catabolism of tyrosine beyond a certain point.

Hyperproteinemia

Hyperproteinemia refers to a plasma protein concentration above 8 gm. per 100 cc. It may be real or apparent, depending on the state of hydration of the blood. Real, or true hyperproteinemia is present only when blood volume is normal,

while false hyperproteinemia occurs when hemoconcentration is present.

True hyperproteinemia is invariably due to hyperglobulinemia. Increased globulin levels arise as part of the defense mechanism of the body in response to the presence of antigen. Globulin is apt to be increased, therefore, in any infectious condition. Also, since globulin is considered to be produced by the cells of the reticulo-endothelial system, some of which reside in the liver, hyperglobulinemia may be associated with diseases of both the reticulo-endothelial system and the liver.

Albumin levels rarely exceed 5 gm. per 100 cc. They are usually decreased when globulin is increased. Hypoalbuminemia and hyperglobulinemia may coexist (as in cirrhosis of the liver), and whether the overall blood picture reveals hypo- or hyper-proteinemia depends upon the extent of the changes in both.

Those infectious conditions most often giving rise to hyperglobulinemia are lymphogranuloma inguinale, Boeck's sarcoid, leprosy, kala azar, schistosomiasis, and subacute bacterial endocarditis; it is also found in chronic suppurative infections, malaria, syphilis, tuberculosis, trypanosomiasis and rheumatoid arthritis. Whenever elevated blood protein levels are found, Jeghers and Selesnick *(3)* suggest that the Frei test be given in order to exclude lymphogranuloma venereum, since hyperproteinemia is so often a part of the clinical picture of this disease. Likewise, in subacute bacterial endocarditis, hyperglobulinemia is considered a constant enough feature to be of diagnostic significance.

The incidence of hyperproteinemia in multiple myeloma is about 50% to 60% *(4)*. The highest total protein values ever seen (as much as 16%) have been reported for multiple myeloma. According to some investigators *(3)*, the elevated

plasma protein level is of greater diagnostic value in this disease than is detection of Bence-Jones proteinuria.

In kala azar, hyperglobulinemia is always present, but only in somewhat less than half the cases is it sufficiently increased to counteract the diminished albumin and thereby cause evident hyperproteinemia. Moderate increases in globulin are also seen in lymphoid and myeloid leukemia.

Among the liver disorders, hyperglobulinemia is seen most frequently in cirrhosis. However, albumin is usually lowered to a greater extent, so that total plasma proteins are more often normal or subnormal than above normal.

Apparent hyperproteinemia, as has already been stated, is due to dehydration of the blood. (See p. 50.) A state of hemoconcentration may develop from acute severe diarrhea or vomiting as in intestinal obstruction, cholera and diabetic coma. When vomiting or diarrhea continue for a period of time, however, protein as well as fluid loss occurs, so that there develops a deficit in total plasma protein as well as a fluid deficit. Under these circumstances, the plasma proteins will appear either normal or slightly elevated according to the extent of the dehydration.

The incidence of hyperproteinemia is not high. Cardon and Atlas *(5)* recently found a frequency of occurrence of 1.2% of plasma protein values above 8.5% in over 4,000 random blood determinations. In the 38 cases of hyperproteinemia which were available for study, there were 8 in whom dehydration was considered a factor contributing to the hyperproteinemia.

In addition to quantitative blood determinations, there are several clinical tests which are of aid in the detection of hyperproteinemia. According to Cardon and Atlas, both a positive

Takata-Ara and a positive formaldehyde-gel test are almost invariably associated with hyperglobulinemia. Although the sedimentation rate is increased in hyperproteinemia, there is no direct correlation between the observed increases and either globulin or total protein levels. No particular histopathologic alterations have been discovered in connection with hyperproteinemia or hyperglobulinemia.

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PART THREE

CLINICAL CONDITIONS ASSOCIATED WITH PROTEIN DEPLETION

Having dealt with the pathological phenomena of altered protein metabolism, we are here concerned with those specific clinical problems arising from or leading to the deficiency states.

Clinical problems, of course, are the primary concern of the practicing physician. It is too well recognized, however, that frequently great delays are encountered in the applications of findings from the research laboratories to the problems of the clinician. At the present time this is not so in the field under consideration. Much of our newer fundamental knowledge of protein metabolism and therapy derives from clinical subjects rather than experimental animals. Here the clinic is in many instances the prime mover, not merely the testing ground.

Section One . . . NUTRITIONAL EDEMA

Only one of all the clinical conditions in which protein levels are lowered – nutritional edema – is recognized as a true deficiency disease. Also called war edema, hunger swelling, famine edema, alimentary dropsy, hunger edema and prison edema *(1)*, these titles graphically designate some of the situations and circumstances which lead to this condition.

The primary etiological factor in nutritional edema is continued inadequate nitrogen intake, possibly complicated by insufficient caloric intake *(2)*. Investigation of the diet usually reveals a high proportion of foods rich in carbohydrate and water, and a minimal content of proteins.

The symptoms of this protein deficiency disease relate to depletion of both plasma and tissue protein. Edema is the cardinal symptom of plasma protein reduction. Though it varies in extent and intensity, it usually appears first about the feet and ankles, then progresses in more severe cases to the abdomen (ascites), and finally, throughout the body (anasarca). Pulmonary edema is particularly dangerous because it reduces resistance to pneumococcic infection. If the patient remains in bed, edema of the face, eyelids and sacrum is apt to appear. The tendency to involvement of the lower extremities in ambulant cases, and the face in bedridden cases, testifies to the contributory role of hydrostatic pressure *(3)*.

In many cases, the appearance of edema brings the patient to the physician. Although edema is indeed the most striking feature, many other factors besides plasma protein levels may modify its extent or determine its appearance. Therefore, diagnosis of protein deficiency disease may be sometimes overlooked in cases of 'nutritional edema without edema' *(4)*. In such cases, the musculature of the patient is steadily consumed. Mus-

cular wasting represents tissue protein depletion.

Since tissue protein depletion is proportionally greater than plasma protein depletion, weakness, fatigue and a disinclination toward exertion are even more common and earlier manifestations of protein deficiency than edema. Work is accomplished with increasing difficulty, and efficiency is below par. In edematous individuals, the muscular atrophy may or may not be apparent depending upon the extent of the swelling. However, when edema is overcome by therapeutic measures, the extreme emaciation of the patient is readily discernible.

Other irregularly occurring symptoms are bradycardia *(5)*, lowered basal metabolism *(6)*, and mild mental depression. Minot *(7)* has reported an interesting case in which fatigue and mental depression were the primary symptoms. His patient complained, "I have totally forgotten how to smile".

Anorexia and vague indigestion are both causative and resultant factors in nutritional edema *(8, p. 244)*. Often patients report loss of weight preceding the appearance of the edema. Polyuria and nocturia have been mentioned also *(6)*.

The symptomatology is entirely non-specific. Although edema is the essential feature of the disease, yet it may be due to causes other than protein deficiency. Clinical diagnosis depends upon ruling out edema of other origin (cardiac, renal, pregnancy), and finding lowered blood proteins. In early cases, the demonstration of the latter in itself is considered sufficient evidence if uncomplicated by liver disease, or excessive loss, as in albuminuria *(8, p. 245)*.

The hypoproteinemia of simple nutritional edema is nearly always the result of hypoalbuminemia. Bruckman and Peters *(9)* found the critical range for the development of edema in their cases to be 3 to 4 gm. of albumin per 100 cc. Globulin is affected less by dietary deficiencies, but in the more severe

cases, it may also be at low or below normal levels. However, when infection is superimposed upon nutritional edema, globulin values may be elevated. Albumin is rarely elevated. When that exists, dehydration of the blood should be strongly suspected.

Treatment of nutritional edema requires introduction into the diet of the deficient component – protein. High protein, high caloric diets are, therefore, recommended. Animal protein appears to be more efficacious than vegetable protein; some observers *(10)* have even reported that 2 gm. of the latter are necessary to equal the reparative effect of 1 gm. of the former. In view of the supplemental action of complete and incomplete proteins towards each other, a mixed protein diet is favored. In some cases, merely increasing the caloric value of the diet has cleared up the edema, an example of the protein-sparing action of carbohydrate. Nevertheless, increasing the protein of the diet is a more direct therapeutic measure, since, although body protein loss is retarded by carbohydrate, only protein can supply nitrogen for replacement purposes.

Uncomplicated protein deficiencies rarely occur in humans; nutritional edema is also very often associated with avitaminoses and anemia *(11)*. As was pointed out previously, nutritional edema may occur secondarily to other chronic diseases rather than as a primary condition itself. Chronic diseases furnish the background for many of the cases encountered during periods of ample food supply. Gastrointestinal disturbances such as dysentery, fistulas, colitis, peptic ulcer, etc., are often associated with hypoproteinemia. Anorexia, cardiac disease, tuberculosis, anemia and alcoholism, for example, may lead to reduction of plasma proteins. Infants and children are particularly prone to develop hypoproteinemia and edema when given faulty diets *(12)*.

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Practically, anemia may be considered a deficiency of hemoglobin, of red blood cells, or of both. That the formation of hemoglobin requires ample protein as well as iron supplies has already been demonstrated and discussed. (See p. 31.) Mere restatement of the fact that the protein, globin, comprises 95% of the hemoglobin molecule is indicative of the protein need in hemoglobin synthesis.

The stroma of the red blood cell, that is, the network within which hemoglobin is deposited, is made up of lipids and proteins. Its formation is of "equal importance with that of hemoglobin since deficient stroma in red cells makes adequate transportation of hemoglobin impossible. Inadequate stroma also causes the erythrocytes to be unduly fragile so that excessive numbers are destroyed by the normal amount of battering to which they are subjected" *(1)*.

Besides the need for building materials, vitamins, thyroxin, and other substances, normal erythrocyte formation requires the presence of an erythrocyte maturing factor. This substance, stored in the liver, is necessary for the complete maturation of the red blood cell; in its absence immature nucleated cells appear in the blood stream. Erythrocyte maturing factor is the product of the interaction of intrinsic factor from the gastric juice, and extrinsic factor present in certain foods. Intrinsic factor may be an enzyme, while extrinsic factor, which is found in muscle meats particularly, is considered by many investigators *(2)* to be protein in nature. In disorders of blood formation, protein depletion may thus be of etiological significance, and protein administration of therapeutic value.

The anemias of decreased blood production may be classified into the macrocytic types in which red cell formation is

impaired, and into the hypochromic types in which hemoglobin synthesis is deficient. That macrocytosis may have an etiological basis in hypoproteinemia was shown recently by Bethell and Rottschaefer *(3)*. In a study of over 100 anemic individuals, all those having a serum albumin of less than 4 gm. per 100 cc. were found to have macrocytosis. It was concluded that an increase in red cell size may be due to hypoproteinemia as well as developmental defects of the blood-forming tissues (*e.g.*, erythrocyte maturing factor deficiency). Macrocytic anemia of pregnancy has been shown to be due, in many cases, also to low protein intake. (See p. 93.)

Hypochromic anemia arising as a result of decreased protein intake has been demonstrated experimentally in laboratory dogs *(4)*. After 80 days on a low protein diet, 22.2% of the nitrogen lost from the circulation was hemoglobin nitrogen. When considered in terms of absolute quantity of protein removed from the circulation, six times as much hemoglobin as serum albumin was lost. Part of the hemoglobin depletion may also have been due to a lack of structural material for erythrocyte formation.

Excellent clinical examples of hypochromic anemia resulting from deficient protein intake are those cases of nutritional edema reported by Youmans *(5)*. In nearly all of his cases, hypochromic anemia was present – obviously a situation in which protein intake was inadequate both for maintenance of fluid balance and for normal blood formation.

There is, then, considerable evidence to show that protein depletion is commonly associated with anemia. Protein intake is apt to be low for long periods preceding the appearance of the disease. The dietary history of anemic patients often reveals this. Murphy *(6)* states, "the patient with pernicious anemia is likely to have developed a capricious appetite with rather char-

acteristic likes and dislikes. Protein food, particularly meat, is disliked or poorly tolerated by many and is consequently eliminated from the diet. In the place of meat may be substituted dairy products, including often large quantities of fat. Others for years have taken little else than a starchy food such as bread washed down with tea or coffee." Clearly, a distaste for meat among anemic individuals is a rather common symptom *(7)*.

In those anemic patients having hypochlorhydria or achlorhydria, the extent of impairment of protein digestion is proportionate to the degree of anacidity. Though they may consume a normal amount of meat protein, these individuals cannot digest proteins completely and therefore their effective protein intake may be as low as the actual intake of those who dislike meat.

One of the most extensive investigations of the plasma protein levels in pernicious anemia *(8)* revealed values below normal in 76% of the patients. The extent of the decrease in plasma protein concentration was considerable – as much as 40% to 50% of normal in some instances. Additional reports made by other investigators *(9; 10)* are in general agreement as to the high incidence of hypoproteinemia in pernicious anemia. In other macrocytic anemias and in the hypochromic anemias, low blood protein levels have also been frequently encountered *(11-16)*.

Edema occurs in over 50% of pernicious anemia patients. If all pernicious anemia patients were followed to death, edema would be almost a constant symptom *(10)*. No direct correlation has been found between the edema of anemia and reduction of plasma protein levels or osmotic pressure *(14)*. Anemia *per se* is believed to be conducive to water retention *(17)*. Perhaps anoxemia, resulting from the anemia, increases capillary permeability to proteins *(15)*. At any rate, the frequently decreased

plasma protein levels encountered in anemia cannot but accentuate the edematous tendency. Edema thus appears at higher plasma protein levels than in simple protein malnutrition. In one series, the critical levels for edema in anemia were found to be 4% for albumin and 5.8% for total plasma proteins. Clearly, plasma protein levels should be kept as high as possible if the edema of anemia is to be held in abeyance.

A striking observation in experimental animals (dogs) made hypoproteinemic and anemic has been recorded by Whipple *(18)*. In those studies it was found that protein, protein hydrolysates, or amino acids invariably yielded more hemoglobin than serum protein. The mechanism for the manufacture of hemoglobin, in the presence of an adequate supply of essential food accessories, seems, then, to have preferential demand on the ingested protein.

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The average protein concentration in the blood of pregnant women is lower than the average level in non-pregnant women. Thus, during the first six months of pregnancy, the serum proteins slowly fall from 7 gm. to about 6.2 gm. per 100 cc. *(1)*. In the last trimester they rise almost to the higher figure, but following delivery, drop again. Several days thereafter, another gradual climb begins, reaching normal non-pregnancy levels about 7 days postpartum *(2)*.

Transient changes which may occur at term are considered due largely to changes in blood volume. The lowered levels noticed earlier in pregnancy indicate changes in blood volume and in total protein content as well, for, in some cases, the reduced blood levels are accompanied by slight alterations of the A/G ratio *(2)*. The needs of the fetus and the subsequent requirements for milk production have been likened to an "internal plasmapheresis" because they draw so heavily on the plasma and reserve proteins of the mother *(3)*. It is these additional demands upon the protein supplies rather than alterations of plasma volume which are of primary importance in causing lowered serum protein levels.

Notwithstanding the normal physiological drop in plasma protein concentration, very frequently blood protein levels may become even further reduced. Abnormally low levels have been reported in association with nutritional edema, the anemias of pregnancy, and the toxemias of pregnancy. A common denominator in all these conditions is low protein intake.

The National Research Council has recommended increased daily intake of protein to the extent of about 40% during the latter half of pregnancy and about 67% during lactation. Recent analyses of the diets of a mixed group of 514 pregnant

women *(4)* revealed startlingly low protein intakes; 86.9% of the women ingested less than the recommended amount, while the average protein intake for the entire group was 22% below the standard. Another study of dietary intakes *(5)*, this one of 225 unselected patients, revealed that only 3% of the negro, 2% of the white charity and 12% of the private patients consumed the recommended amounts of protein. In 30% of the negroes, 20% of the white clinic patients, and 10% of the private patients, protein intakes were dangerously low. A high incidence of protein deficiencies among pregnant women is readily apparent. The extent of the deficiencies among private patients makes it clear, also, that the cause is not wholly economic.

Some women who ingest ample amounts of protein will develop nitrogen deficiency nevertheless. More or less persistent emesis plays a role in many cases. If this progresses to hyperemesis gravidarum, the effective protein intake becomes chronically low, leading, within a short time, to extreme protein depletion. However, since dehydration also results from pernicious vomiting, plasma protein determinations often indicate apparent hyperproteinemia instead of hypoproteinemia. (See p. 50.)

Digestion is usually impaired at some time or other during pregnancy. Achlorhydria seems to be a rather prevalent condition at this period. One investigation *(6)*, for example, revealed that 75% of the pregnant women examined did not secrete normal amounts of free hydrochloric acid or pepsin during more than half the period of pregnancy. Another report *(7)* showing only 25% hypochlorhydria and achlorhydria does not contradict the 75% figure because a majority of the patients in this later study were in the last month of pregnancy, a time when those in the first group also showed a rise in acidity. Whatever the exact incidence, any reduction of digestive

power at a time when heightened digestive power would be advantageous cannot but inhibit optimal nutrition.

The significance of low protein intake in pregnant or lactating women should not be underestimated. Fetal growth demands an uncompromising definite quantity of protein for muscle, organ and bony tissue synthesis. Even larger quantities of nitrogen are required for milk production. Superimposed, then, upon the normal maintenance requirements of the mother are those of the fetal parasite and, postpartum, of lactation. When the protein intake does not satisfy this dual demand during pregnancy, the fetal need assumes priority, and the reserve proteins of the mother are consumed *(8)*. Should the discrepancy between supply and demand continue, depletion of the reserve depots and hypoproteinemia ensue.

Furthermore, calcium absorption may be hindered. This has been mentioned before (see p. 68), but it deserves reiteration in the light of the greatly increased calcium need during pregnancy and lactation. Metabolic studies on human beings *(9)* have definitely established that augmented protein intake results in increased absorption of calcium from the intestine. At a constant level of calcium intake, the percentage of calcium absorption appears to be proportional to the percentage of protein in the diet.

A carefully controlled study of the effect of "good" and "poor" diets on pregnant women has been reported recently *(10)*. Poor diets, defined as yielding 1600-1900 calories, averaging 60 grams of protein, and also deficient in vitamins and minerals, were studied in 120 cases. Good diets, in which supplementation brought the caloric content to 2600 calories and which raised the protein level to 100 grams and added adequate amounts of vitamins and minerals, were given to 90 cases.

Impartial ratings of the course of the pregnancies by attending obstetricians, plus statistical data concerning the incidence of illnesses and complications, revealed striking differences between the two groups. Only 6% of the women on the good diet had a "bad time" during the course of pregnancy as against 34% of the women on the poor diet. The percentage of babies having a poor record the first two weeks was 15% for those born of mothers receiving a poor diet and 0% for those whose mothers ingested the supplemented diet. The infant mortality rates at 6 months were 3% and 0% respectively.

These studies show that the influence of the dietary intake during pregnancy is far reaching in its effects. (Also see *(11)*.) Besides reducing the complications of both pregnancy and labor, satisfactory pre-natal diet favorably affects the health of the infant for at least six months after birth.

In view of the greater protein need during pregnancy and lactation, low protein intakes should cause nutritional edema sooner than in non-pregnant individuals. Arnell and Guerriero *(12)*, who have made a detailed study of nutritional edema in pregnancy, state that this disease is actually encountered more often on the obstetrical than on the medical services of most hospitals. Edema of the vulva is mentioned as a particularly distressing, yet somewhat helpful diagnostic symptom. In their cases of nutritional edema of pregnancy, the average protein level was 4.20%, the average albumin level was 2.03%, and the average protein intake was 0.54 gm. per kg. of body weight. (The National Research Council recommends about 1.5 gm. per kg.)

Edema occurs with a frequency of about 64% in pregnancies *(13)*. The edema may be mild and due solely to increased venous pressure, or may be serious, associated with anemia and toxemia. Differential diagnosis to detect the severe cases is essen-

tial, and this requires investigation of red blood cell count, hemoglobin, plasma proteins, blood pressure and urine *(14)*.

Anemias of Pregnancy

Anemias of pregnancy due to nutritional inadequacy follow the same pattern as the nutritional anemias. (See the preceding section, pp. 84 to 88.) Generally, figures as to incidence in pregnancy reveal a preponderance of the hypochromic over macrocytic anemias, but perhaps this merely reflects the greater frequency of iron deficiency *(12)*. The accessory role of lowered gastric activity in the production of hypochromic anemia has been emphasized *(15)*. Assuming that the ability of the stomach to secrete acid serves as an index of general gastrointestinal tract function, the greater incidence of hypochromic anemia of pregnancy may be explained partly on the basis of hypochlorhydria. Impaired digestion of protein and utilization of iron in conjunction with lowered intake and increased need undoubtedly account for most if not all of the hypochromic anemias of pregnancy.

The blood picture of macrocytic anemia of pregnancy resembles that of pernicious anemia. Whether macrocytic anemia of pregnancy is due to deficiency of the extrinsic, the intrinsic, or both factors has still to be determined. Some *(16)* among those who believe that lack of extrinsic factor is the principal etiological agent feel that vitamin B complex is the important element. On the other hand, both experimentally in rats and clinically in human beings, the cause of macrocytic anemia of pregnancy has been assigned to insufficient protein *(17)*. "Anemia of pregnancy associated with inadequate food intake is a true macrocytic anemia and in its more severe manifestations is probably identical with the so-called 'pernicious anemia' of pregnancy. Although it has been reported that a deficiency of vitamin B complex may lead to such anemia our results indi-

cate that a closer correlation exists between the incidence of macrocytic anemia and inadequate intake of animal protein than between such anemia and deficiency of either thiamin, riboflavin or the pellagra preventing factor" *(18)*.

Fifty-four percent of a group of 158 pregnant women studied by Bethell were found to have true anemia, that is, a red blood count of less than 3.5 million, or a hemoglobin value of less than 10 grams. The etiological factors were lack of iron and insufficiency of animal protein. When iron therapy fails to cure an anemia of pregnancy, as so often happens, correction of other dietary inadequacies may solve the problem. Insistence upon optimum diets for normal pregnancies and supplemented diets in complications of pregnancy should result in fewer cases of anemia during this period.

Pre-eclampsia and Eclampsia

Excluding the toxemias due to pre-existing hypertension or vascular-renal disease, there remain the eclampsias. Eclampsia has been divided into the following chronological stages: mild pre-eclampsia, severe pre-eclampsia, and eclampsia. Obstetrical attention has been focused purposely upon the prevention and treatment of the pre-eclampsias, for only by controlling these will the incidence of eclampsia itself be reduced. The main signs of pre-eclampsia are sudden gain in weight due to water retention, albuminuria, and a rise in blood pressure.

The role of hypoproteinemia in the etiology of the edema of the eclampsias has been considered by several workers *(19)*. Foremost among these is Strauss *(20)*; his average figures for albumin concentration are as follows: in normal women, 3.56%; in pre-eclampsia, 3.1%; and in eclampsia, 2.6%. These changes in albumin concentration are reflected in decreased total plasma protein and in decreased osmotic pres-

sure. The decrease in osmotic pressure undoubtedly accentuates the normal tendency to water retention brought about by increased venous pressure.

Other investigators *(21; 22; 13)* besides Strauss have confirmed the lowering of the plasma protein level in eclampsia. In one series *(23)* the albumin level of the control group was 4.27%; in the toxemic patients it was 3.87%. Bibb *(24)* considers this reduction a prognostic indication, for he says, "The finding of a low serum protein value in a woman in the last trimester of pregnancy is often a warning of impending toxemia, especially if the protein has been normal earlier".

The factors bringing about lowered plasma proteins in toxemia are not always explained by inadequate protein intake since acute toxemia and hypoproteinemia have been noted in women with adequate protein intake and previously normal plasma protein concentration *(24)*. However, low protein intake is probably the chief contributing factor; Strauss *(20)* has estimated that the daily protein intake in 20 toxemic patients whose dietary history he ascertained was less than 50 grams. Eighteen of these women had consumed little meat or other protein foods for years preceding their pregnancy. Six of them had also had severe nausea and vomiting early in pregnancy.

Despite the rather general occurrence of hypoproteinemia in pre-eclampsia, some investigators have advanced the opinion that it plays but a secondary etiological role. However, they do concede the significance of hypoproteinemia in the accentuation of symptoms or the precipitation of eclampsia. Its prevention or early correction would therefore seem to be a rational method of combating one feature of this form of toxemia. Maintenance of adequate nutrition with emphasis upon sufficient protein intake has been advocated for the prevention of hypoproteinemia and the edema subsequent thereto *(25, p. 42)*. In the pre-

liminary series reported by Dodge and Frost *(23)*, none of the patients receiving 90 or more grams of protein daily developed toxemia. Definitely, the trend in pre-natal dietary advice aimed at the prevention of hypoproteinemia and toxemia is toward ample protein intakes.

Treatment of pre-eclampsia has centered mainly about salt restriction. A daily diet of 1500 cc. of skimmed milk has been considered one of the simplest means of accomplishing this *(26)*. However, no amount of salt restriction will correct hypoproteinemia, and even though salt restriction may sometimes correct edema in the presence of mild hypoproteinemia and toxemia, the underlying hypoproteinemia still will predispose the patient toward further recurrences unless treatment includes high protein intakes. Strauss *(20)* has reported treating one series of toxemic patients by means of high protein diets containing 260 gm. a day. Weight loss soon was observed, progressing until complete disappearance of edema, usually within two or three weeks. Coincidentally, arterial blood pressure began to fall. Other symptoms of impending eclampsia (headache, visual disturbances, etc.) also abated. Albuminuria either was unchanged or decreased.

The use of high protein diets as an important accessory measure in treating pre-eclampsia is being regarded with increasing favor by clinicians. Albuminuria is now considered an indication for, rather than a contraindication to, high protein diets. In one of the country's most famous maternity hospitals, all pre-eclamptic patients receive 145 gm. protein daily, after a preliminary 24 hour period on an exclusive milk diet *(25, p. 351)*.

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Section Four . . . PROTEIN DEFICIENCY STATES IN PEDIATRICS

Serum protein levels in infants and children up to the age of two years are lower than the normal for adults. The average for infants up to three months is 5.5% for total proteins, 3.6% for albumin and 1.8% for globulin, while for those children from three months to two years the average levels are 6.2% for total proteins, 4.3% for albumin, and 1.9% for globulin *(1)*. In premature infants, blood protein values are even lower *(2)*. Globulin values are unusually small when compared to those of the older population, and it has been suggested that this may be due to lack of the usual (immunologic) stimuli giving rise to globulin production in adults *(2)*, since globulin values rise readily enough when infants become ill with infectious diseases.

Younger children are especially susceptible to the development of nutritional edema because of their low blood protein levels. This deficiency disease assumes particularly grave significance when it occurs in children because of the child's relatively greater need for protein for growth and development (cf. Table I) *(3)*. The following have been given as the protein requirements per kilogram of expected body weight for age *(4)*: breast-fed infants, 2 grams; artificially fed infants, 3.5 grams; school children, 2.5 grams; and older children, 2 grams. In infants, milk, of course, is the sole source of dietary protein, whereas in older children the milk is supplemented by meat, eggs, fish and cheese.

Under certain circumstances, the protein intake of children may be inadequate *(3)*. Besides those born of economically poor parents, there are many with anorexia or capricious appetite who eat carbohydrate-rich foods to the exclusion of all other kinds. Not infrequently allergy to milk is encountered. More-

over, certain digestive diseases seen in childhood bring about protein deficiency through vomiting and diarrhea. Thus, in chronic dysentery, colitis, and celiac disease, the amount of protein nitrogen absorbed into the circulation is nearly always insufficient for the child's needs; hence, the child does not grow, becomes apathetic and eventually hydropic.

The incidence of mild protein deficiencies in children is probably much greater than is realized. One recent investigation of the albumin levels of supposedly-well children in a Tennessee community *(1)* revealed a mild chronic low-grade protein deficiency. These children, besides not being in optimum health, are predisposed towards the development of infections and edema.

That infants and children can absorb and utilize the nitrogen of protein hydrolysates has been shown by a number of workers *(5-7)*. The usefulness of such preparations in the treatment of underweight youngsters with anorexia has also been demonstrated *(8)*. Children with nephrosis *(9)*, glomerulonephritis, or acute gastrointestinal disorders *(10)* will show positive nitrogen balances when given adequate dietary supplementation with protein hydrolysates. Another promising indication for protein digest therapy is the existence of pronounced allergy to protein foods, especially milk and milk products *(11)*.

The remarkable reduction in infant morbidity and mortality which has been achieved through control of the infectious diseases may be further reduced, it is hoped, through intelligent application of the advancing science of nutrition. The newer knowledge of normal and abnormal nitrogen metabolism, coupled with the availability of effective therapeutic weapons, will undoubtedly enable the pediatrician to contribute in no small way to the development of sturdier and healthier children.

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Since deamination of amino acids and production of blood proteins (prothrombin, fibrinogen and albumin, particularly) take place in the liver, disturbances of this organ often are accompanied by disturbances in protein metabolism. In fact, hypoalbuminemia is present at some stage in every case of chronic advanced liver disease and in most cases of obstructive jaundice *(1)*. Most of the investigations of plasma protein levels in liver disease have been concerned with cirrhotic conditions, and for this reason, the following discussion deals mainly with cirrhosis. Generally speaking, alterations of blood proteins similar to those seen in cirrhosis occur in the other liver diseases – arsphenamine hepatitis, catarrhal jaundice, hepatosplenomegaly, chronic hepatitis, cancerous hepatitis and amyloidosis – except that they are usually not as extensive.

Aside from the cirrhoses caused by toxic agents, there remain a large percentage of cases (*i.e.*, portal cirrhosis) in which the exact etiological factor is obscure. The concept that such cirrhoses are deficiency diseases is steadily gaining favor *(2)*. A history of alcoholism is obtained in somewhat over 50% of the cases of portal cirrhosis, but since only 5% of alcoholic addicts develop cirrhosis *(3)*, and since prolonged alcohol administration *per se* has not produced cirrhosis experimentally, the association cannot be a direct one *(4)*.

Fatty livers, associated with liver damage, have decreased resistance to toxins. Cirrhosis often follows fatty degeneration of the liver *(5)*. That ingested protein is lipotropic has been demonstrated by the observation that the amount of fat present in the liver is inversely proportional to the amount of protein, as well as carbohydrate, in the diet *(6; 7)*.

Experimental cirrhosis of the liver in rats has been produced

many times by means of low protein diets *(8-10)*. Casein not only will prevent the development of this type of cirrhosis *(11)* but it will also cause marked improvement and regeneration of liver cells once cirrhosis is established *(12)*. The action of casein has been traced to its content of cystine and methionine. The latter amino acid, it is thought, is converted *in vivo* to the lipotropic agent, choline. However, excessive amounts of cystine promote fat deposition. The positive lipotropic effect of proteins must therefore be due to a preponderance of the preventive factors over those promoting fat deposition.

Besides the experimental evidence obtained from studies of fatty liver infiltration and degeneration, there are several pertinent clinical indications. Strongly suggestive of nutritional deficiency is the high concomitant incidence of peripheral neuropathies and anemias *(13; 14)*. Also, in view of the known high incidence of avitaminoses among alcoholic addicts, it is not surprising that abnormal diets have been found to be secondary to alcohol as factors in cirrhosis *(4)*.

Thirteen cases of alcoholic cirrhosis of the liver examined by Patek *(15)* showed rather consistent malnourishment, especially with regard to vitamin B complex. In Ceylon, Syria, India and China, countries where alcohol consumption is practically negligible, dietary deficiencies of proteins and vitamins among cirrhotic patients are the rule rather than the exception *(16)*. Encouraging results now being obtained with the newer dietary treatment (see p. 104) furnish perhaps the most impressive evidence for the assignment of an etiologic, or at least predisposing, role to dietary deficiencies. Whatever the specific etiological basis for cirrhosis, if there is such a thing, it is apparent that alcohol plus inadequate food intake, or improper food intake itself, will predispose to liver damage.

Once cirrhosis of the liver becomes established, definite

clinical signs such as ascites, jaundice, edema, etc. appear. Ascites has been observed in 78% of the cases; edema in 61.1% *(16)*. Hypoproteinemia appears to be the main cause of water retention *(17)*. Not to be minimized, however, is the role played by portal vein hypertension in accentuating and directing the dropsical tendency to the abdomen.

As was already mentioned, blood protein values are rather consistently altered in cirrhosis. Most often the albumin concentration is reduced. Globulin, if abnormal, is raised rather than lowered. In one clinical study *(18)*, 14 out of 16 cirrhotic patients were discovered to have plasma protein levels below 6 gm. per 100 cc. Ninety-six percent of the cases in another group *(19)* had albumin concentrations below 4 gm. per 100 cc., while 83% of them also had elevated globulins with concentrations above 3 gm. per 100 cc. Widespread confirmation of these findings *(20-22)* and the demonstration of a reduction in osmotic pressure corresponding to the degree of hypoalbuminemia *(23)* have not only established a cause for the ascites and edema of cirrhosis but have also aroused considerable interest in the etiology of the hypoproteinemia itself.

The hypoalbuminemia of liver disease may be due largely to inadequate protein intake. Even with ample nitrogen intake and the establishment of a positive nitrogen balance, however, many cases continue having low blood albumin levels *(24)*. Though often considerable, protein loss in ascitic fluid is not necessarily associated with hypoproteinemia, and most observers feel that it accounts only partially for the low levels of albumin *(25; 26; 18)*. Therefore, it is concluded that albumin synthesis is impaired in these patients. Since the liver is considered to be the main site of albumin formation, the implication with regard to impairment of liver function as a cause of hypoalbuminemia is fairly direct. The rise in globulin has not yet been clarified. When concomitant infection is present,

hyperglobulinemia is explainable on the basis of the role of serum globulins in the immune mechanisms of the body.

In cirrhotic patients, hypoalbuminemia without blood protein loss and in the presence of adequate nitrogen intake indicates impaired albumin synthesis by the liver. Plasma protein determinations are useful, therefore, as diagnostic tests for liver function in these cases *(22; 27)*. In the opinion of some observers *(14)*, "The serum protein determinations appear to be the most constant and perhaps earliest means of determining the severity of liver damage, as borne out by the results in comparison with other liver function tests". According to these investigations, functional liver impairment was detected by plasma protein determinations in 100% of 24 proved cases of cirrhosis, in contrast to 83% detection by bromsulfalein or glucose tolerance tests.

Serum albumin determinations are also of prognostic value. Those patients having ascites have lower albumin values than others. Clinical improvement is associated with a rise of albumin toward normal. According to a recent report *(28)*, about 70% of the patients having serum albumin concentrations above 2.5% at the time of admission survived. If the albumin level is below 2.5%, the prognosis is grave.

Modern treatment of cirrhosis recognizes the significance of dietetic factors. High protein, high vitamin, high caloric diets are prescribed. Previously, when only the carbohydrate intake was considered valuable in maintaining liver cell integrity, high carbohydrate intake and the administration of diuretics composed the accepted routine. At the Mayo Clinic, comparison of this former therapeutic regime with the one now used (350-500 gm. carbohydrate, 110-145 gm. protein, plus vitamin supplements and liver extract) suggests that the newer treatment yields much better results *(3)*. To date, reports indicate

that, whereas 30.7% of the patients showed "satisfactory results" from the former treatment, "excellent results" were obtained with 44% of those receiving the newer therapy *(29)*.

Patek and Post *(19)* treated a series of 54 patients with a highly nutritious diet containing 114 gm. of protein and supplemented with 50 gm. of brewer's yeast daily. They compared the survival of this group with a control group of 386 patients. The survival rate one year after the onset of ascites was 57% in patients treated with high protein, high vitamin diet, whereas only 39% of the controls lived. At the end of the second year, the survival rate was 45% for the diet group and 21% for the controls. "In addition to an increased period of survival, there are signs of general bodily improvement and presumptive evidence of arrest of the disease process. In a significant number of cases there has been disappearance of ascites, edema, jaundice and of vascular spiders." Laboratory tests of liver function also reflected the clinical improvement. Perhaps this new therapeutic rationale, directed toward high protein and vitamin as well as high carbohydrate intakes, indicates the way to ultimate victory over a disease once considered invariably progressive.

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Left untreated, liver tissue damage invariably becomes aggravated. Even adequate nitrogen intake may not bring about an initially increased production of plasma proteins. It will, however, produce positive nitrogen balance, indicating a general restoration of cellular parenchyma. If a sufficient number of liver cells are regenerated, improved functional capacity should be expected. In view of the enormous safety factor of the liver, it may be hoped that even advanced liver diseases may be arrested at a point where adequate functional regeneration will occur. Although the most effective dietary balance

may not yet be established, no patient with liver disease should be deprived of the opportunity to benefit from the administration of high protein, high vitamin, high caloric diets.

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Section Six . . . BRIGHT'S DISEASES OF THE KIDNEY

Bright's diseases of the kidney have been subdivided into three supposedly distinct clinical syndromes. Some confusion has arisen, however, from duplication and overlapping of terms. Thus, what Volhard and Fahr *(1)* called glomerular nephritis, nephrosis and nephrosclerosis, Addis *(2)* referred to as hemorrhagic nephritis, degenerative nephritis and arteriosclerotic Bright's disease. Both classifications will be used interchangeably here. Although some clinicians believe that the nephritic and nephrotic syndromes represent but stages in the progression of a single disease, and although both syndromes may be observed simultaneously in the same patient (for example, the nephrotic stage of nephritis), they will be considered individually in accordance with the generally accepted distinctions.

Nephrosclerosis

In the nephrosclerotic syndrome, plasma protein levels are usually normal *(3)*; inadequate intake is considered responsible for hypoproteinemia when it occurs during the course of the disease, although plasma volume changes may affect the protein concentration terminally.

Glomerular Nephritis

Patients with hemorrhagic Bright's disease – the glomerulonephritis of Volhard and Fahr – pass, in variable order, through several or all of a number of phases, from the acute to terminal or healed, via either one or both of latent and chronic active stages. Cardinal features of this inflammatory disease are proteinuria, hematuria, edema, hypertension and nitrogen retention.

The edema of glomerulonephritis may have its origin in one

or more of three circumstances – increased capillary permeability, cardiac failure, or hypoproteinemia *(4)*. In the acute stage, increased endothelial permeability develops from the presence of toxins elaborated usually by streptococcus hemolyticus. Cardiac edema, on the other hand, appears as a terminal manifestation *(5)*.

Reduction of plasma proteins occurs in nearly all but the very mild cases of hemorrhagic nephritis *(6)*. When it appears during the acute phase, the edema initiated by increased vascular permeability persists rather than subsides. Of 23 acute cases reported by Van Slyke *et al. (7)*, 19 showed lowered blood proteins; among these were 7 with plasma albumins under 2.2%, a sign of prognostic significance in that 5 patients in this group developed chronic or terminal nephritis. On the other hand, of the 12 patients with albumin values above 2.2%, only one progressed to these stages. The demarcation point was related to albumin rather than to total plasma protein because globulin levels, though often raised, change less consistently.

Remission to the latent stage of glomerulonephritis is accompanied by a rise in serum proteins to, or almost to, the normal range. During the chronic active stage of the disease, however, plasma albumin values are almost always lowered, usually below the 2.5% level. When the terminal phase is reached, a sufficient number of the cases show a rise in the blood proteins to reduce the frequency of hypoproteinemia at this stage to 50%. Hemoconcentration has been shown to play a part in causing this terminal increase in blood protein concentration *(5)*. As was mentioned before, edema of the final phase is at least as often due to cardiac failure as to hypoproteinemia *(7)*.

With regard to the causation of the hypoproteinemia of

hemorrhagic nephritis, one significant factor is proteinuria, especially during the chronic active period, the so-called nephrotic stage of glomerular nephritis. Not uncommon is an excretion of 10 to 20 grams of protein per day. High urinary A G ratios (between 2 and 5 to 1, or even as high as 10 to 1) besides indicating a greater loss of albumin than globulin, explain both the greater reduction in this plasma protein fraction and the proportionate lowering of the colloid osmotic pressure *(8-10)*.

Proteinuria, however, is not deemed sufficient to account entirely for the observed blood protein levels *(11)*. The marked tissue wasting which almost invariably accompanies nephritis suggests another factor. Using such criteria as obvious emaciation, weight loss, and records of chronic dietary inadequacy, Peters and his co-workers *(12)* found a surprisingly large incidence of malnutrition in their nephritic patients – so much as to lead them to conclude that the serum protein deficiency is due mainly to exhaustion of body protein stores. This view of protein depletion as an important cause of hypoproteinemia in nephritis is shared by practically all authorities *(13; 14, p. 679)*. Though probably there may be other causes such as impaired ability to synthesize serum proteins *(15)*, 'loss and lack' of protein are undoubtedly major factors contributing to hypoproteinemia in glomerulonephritis.

The dietetic management of patients with hemorrhagic nephritis has long been a controversial subject. Adherents of low protein intakes base their opinion upon the apparent kidney damage seen in animals fed high protein diets and upon the increased functional burden to the kidney in excreting non-protein nitrogenous metabolites, principally urea. The former reason is not regarded as particularly significant since kidney damage appeared only when the animals were fed excessively high protein intakes. Furthermore, similar damage

has not been reported in humans. It has not been seen among the Eskimos, whose protein ingestion constitutes 40% of their total caloric intake, nor among those Arctic explorers who subsisted upon exclusive meat diets for several years *(16)*. Even if excessive proportions of protein should definitely be proved harmful to man, this should not be a contraindication to normal protein intakes. Certainly it should not lead to a regime of extreme protein restriction *(17)*.

With respect to the possible functional burden imposed upon the kidney by protein metabolites, it should be pointed out that not all ingested protein is deaminized to urea and similar end products. Food nitrogen which is retained for the formation of body and plasma proteins, for hemoglobin, bile acid, hormone and enzyme synthesis, imposes no strain upon the excretory mechanism. In the opinion of most authorities, protein restriction is necessary – if at all – only when kidney function is severely impaired and retention uremia imminent *(18)*. Since nitrogen retention does not even begin to take place until the blood urea clearance falls to 50% of normal, and is constantly present only when below 20% of normal, this test may be of assistance in determining the optimum nitrogen intake for a particular patient at a particular time. Peters and Van Slyke *(14, p. 689)* have even mentioned the remarkable vitality and vigor displayed by several nephritics whose urea clearances were even less than 20% of normal but whose diets were unregulated. They believe, "that we have as yet no criterion for the renal state that requires protein restriction, and that the condition of nephritic patients in general would be happier, the progress of their disease no more rapid, if their diets were regulated in accordance with the usual metabolic requirements of persons of their size, age and activity, with due regard to the state of their digestive organs and their general condition, and without regard to the logical but clinically unproved as-

sumption that protein restriction should accompany retarded urea excreting ability".

Greater clinical improvement has followed the use of high protein diets in glomerular nephritis. For example, a group of patients with hemorrhagic nephritis were given increasing amounts of dietary protein (40 to 200 gm. per day) *(19)*. No deleterious effects upon the course of the disease, extent of hematuria, or rise in blood pressure were observed. In fact, functional capacity as measured by urea clearance improved. The slightly increased proteinuria sometimes resulting from the higher nitrogen intake was not considered harmful, and was taken as evidence of increased serum protein regeneration. On the whole, the patients' general health progressed to the extent that they returned to their normal strength and vitality. In another report *(15)* on nitrogen balances, it is stated that nephritic patients store large amounts of nitrogen when their caloric and protein intakes are sufficiently raised. Increased proteinuria does not appear until several days after institution of supplemental protein – indicating restoration of depleted reserves during the interval.

Forty nephritic children who had been previously receiving low protein diets were put on high vitamin and high nitrogen intakes *(20)*. Under the former treatment the disease process was not arrested and all patients remained chronic invalids, while under the newer treatment definite clinical improvement was observed, progressing even to apparent cures in several instances. There was no increase in blood non-protein nitrogen values as a whole. Weight curves rose, albuminuria tended to decrease, and actually disappeared in three instances.

Up to this point, the evidence cited has demonstrated not only that liberal protein intakes are not harmful, but also that they are of decided benefit. When the effects of a low protein

diet are considered in terms of tissue deterioration within the kidney as elsewhere, in the tendency to anemia, and in the susceptibility to infection *(18)*, it is apparent that there is no justification for low protein intakes except perhaps in cases of extreme nitrogen retention.

The exact amount of protein to be given depends upon the protein need. In nephritic patients the normal requirement is augmented by tissue depletion, hypoproteinemia and proteinuria, while the rate of protein catabolism appears to be considerably speeded up. Only when the protein intake becomes adequate to cover the protein catabolism and the albumin loss will body nitrogen wastage be prevented *(13)*. Some clinicians *(18)* prescribe 75 grams of protein plus the amount lost in the urine as the daily intake. This brings the protein allowance to about 100 grams a day, unless the proteinuria is excessive even for nephritis, in which case 125 grams or more may be required.

Nephrosis

The patient with nephrosis presents three invariable signs – albuminuria, hypoproteinemia, and edema. Lowering of the basal metabolic rate and the presence of lipoidemia are somewhat less common, though still very frequent, characteristics. In consequence of the hypoproteinemia, serum calcium is also reduced *(21)*. Differential diagnosis from hemorrhagic nephritis is based upon the absence of hypertension, hematuria, and renal insufficiency (as determined by normal urea clearance and specific gravity tests). Regardless of whether nephrosis is a distinct pathological entity or a phase of glomerular nephritis, a metabolic disorder or a renal disease *(22)*, it is of particular interest in connection with altered protein metabolism.

Of all the clinical conditions in which hypoproteinemia is a

manifestation, maximum deficits in plasma proteins are seen in nephrosis. Albumin concentration falls to 2% or less. Globulin may be normal or increased; whatever increases do occur are not comparable to the albumin decrease, so that total plasma protein is almost always lowered – and approximately proportional to the albumin reduction. Thus, when albumin values are below 2%, total plasma proteins are usually below 5% *(14, p. 673)*. There is even some interesting evidence *(23; 24)* to show that the serum proteins in nephrosis may differ immunologically and chemically from those in normal serum.

Edema generally appears when blood albumin levels descend to 2.5% and total proteins to 5.5% *(14, p. 673)*. Though preceded by fatigue and weakness, edema is usually the first symptom of nephrosis bringing the patient to the physician. As Fishberg *(25, p. 381)* says, "to the patient it is the disease".

As a prognostic indicator of the course of a nephrotic condition, plasma protein and albumin determinations have been found helpful. With recovery or remission, their concentration rises. They may also rise following the institution of high protein intakes, and be attended by diminution of edema as well as by considerable clinical improvement. Persistence of the albuminuria, however, demonstrates that the underlying abnormality has not been altered.

The hypoproteinemia of nephrosis is due largely to an albuminuria which is far more extensive than in any other disease. Some patients regularly lose 20 grams of protein in the urine daily for long periods of time, and losses of 30 grams have been reported. The A/G ratio of the urine is generally 10 to 1 or higher; albumin thus constitutes over 90% of the urinary protein *(10)*. Other than for the presence of albuminuria, renal function seems unimpaired in true nephrosis, with

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blood urea clearance and non-protein nitrogen normal.

A most interesting advance in our knowledge of the metabolic disturbance underlying nephrosis was recently brought to light by Farr and his associates. Using a relatively new and highly specific method for determining amino acid nitrogen levels in the blood (the ninhydrin method), a characteristic hypoaminoacidemia in nephrosis has been observed. Healthy individuals have an average of 4.5 mg. of amino acid nitrogen per 100 cc. of plasma *(26)* whereas nephrotic children show chronically lowered values of about 3 to 4 mg. per 100 cc. *(27)*. An acute fall in plasma amino acid nitrogen to below 2.5 mg. per 100 cc. occurs during a typical complication of the nephrotic syndrome known as the nephrotic crisis *(28)*. This is an acute febrile episode characterized also by loss of nitrogen and by symptoms of peritonitis with or without bacteremia. Recovery, often as rapid as the dramatic appearance of the attack, is attended by a rise in plasma amino acid content. Attempts at influencing the chronic hypoaminoacidemia by means of oral protein or intravenous protein digest have been unsuccessful. However, the intravenous administration of protein digest has been of distinct benefit during the crisis in that the injected nitrogen was retained and utilized by depleted tissue. This procedure alone, Farr *(29)* reports, reduced the mortality rate in nephrotic patients with pneumococcal bacteremia from 60% to 0% in two years. Greater knowledge of plasma amino acid levels in other diseases as well as in nephrosis may lead, in the future, to impressive therapeutic advances.

Pursuing a chronic course for long periods, with edema subsiding and returning, nephrosis has been a discouraging disease to treat. It is generally recognized, however, that by control of the edema and maintenance of adequate nutrition, nephrotic patients may be kept fairly active and happy. Just as with chil-

dren in the chronic active stage of glomerulonephritis, the protein need in nephrosis is augmented above the normal pronounced tissue depletion, by 'toxic' nitrogen catabolism *(30; 13)*, by albuminuria and by hypoproteinemia. Restoration and maintenance of positive nitrogen balance are imperative for correction and prevention of tissue protein depletion. A sufficient increase in the daily protein intake will establish positive nitrogen balance and cause disappearance of edema. The immediate polyuria which follows greater protein ingestion may be due either to one or more of the following: increased blood volume, increased basal metabolic rate (as a result of specific dynamic action), or the diuretic action of urea *(25, p. 153)*. A sustained rise in plasma protein levels to the normal range may not occur, either because of a more urgent demand for nitrogen by the tissues, an impaired ability to synthesize plasma proteins *(11; 31; 32)*, or continuous albuminuria. However, as Peters and Van Slyke *(14, p. 687)* say, "the accomplishment of an increase of 0.5% may make the difference between complete incapacity and comparative well being".

Epstein *(33)* pointed out the relation between albuminuria, hypoproteinemia and the edema of nephrosis. He advocated diets containing 120 to 240 grams of protein daily. Most clinicians today favor amounts approximating the lower figure. Besides the difficulty in getting patients to ingest more than 125 grams of protein daily, larger amounts may impose such a burden upon the digestive apparatus that gastrointestinal disturbances occur. Possibly Farr's method is best. According to his calculations, 2.5 grams of protein per kilogram of body weight are necessary to secure positive nitrogen balance in nephrotic children *(30)*.

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In Bright's diseases of the kidney, augmented nitrogen metabolism indicates that the nitrogen intake must likewise be

raised if plasma protein levels and tissue nutrition are to be maintained. Nephrosis and nephritis, then, are clinical disorders whose minimum nitrogen requirements are greater than those for the normal state. High protein diets, while not correcting the underlying disorder, will control edema, raise the plasma protein level, provide adequate nitrogenous nutrition, and bring about striking improvement in the well-being of the patient.

Generally speaking, treatment of the nephritides is being based more and more upon the nature of the accompanying metabolic disturbance than upon the type of pathologic change present in the kidneys *(34)*. Accordingly, therapeutic measures to be employed aim primarily at correction of concomitant disturbances in body chemistry and physiology, *e.g.*, hypoalbuminemia, edema, azotemia, acidosis and hypertension.

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Section Seven . . . PROTEIN DEFICENCY STATES IN GERIATRICS

Moderate protein deficiency in aging individuals may be a significant factor in the causation of such subclinical conditions as mild anemia, undue fatigue and lassitude, and lessened vigor *(1)*. Geriatrics, the branch of medicine concerned with the care of the elderly, is interested, therefore, in the correction of protein depletion states at least for the purpose of adding "life to the years, if not years to life".

That protein intake is frequently inadequate in individuals over 60 is well known. Generally, appetite wanes with advancing years, and for some obscure reason, protein foods are among the first victims of this restriction. Difficulty in mastication due to loss of teeth and to new dentures sometimes accounts for reduced meat intake. Perhaps, also, voluntary protein restriction results from impaired digestive ability. Over 40% of the population above age 60 are said to have achlorhydria *(2)*, a phenomenon undoubtedly giving rise to impaired peptic activity and hence, impaired protein digestion.

The fallacy behind protein restriction lies in the fact that protein requirements do not lessen with the advancing years as do caloric requirements. Older people expend less energy, but the dynamic nitrogen exchange within their body tissues continues undiminished throughout life. Caloric intake should be reduced to avoid the hazards of obesity, but protein ingestion should be kept ample so that body tissues will function at peak efficiency.

The importance of ample nitrogen intake in elderly people extends beyond mere provision for structural completeness. The specific dynamic action of proteins, for example, by furnishing additional heat energy, enhances the feeling of well-being so

valuable to the continuation of sound mental attitudes at this period of life. Maintenance of liver function, too, is of no less importance in the care of the elderly than in younger individuals. Possibly of greatest systemic concern, though, is the part played by plasma protein in regulating fluid balance, for reduced blood volume undoubtedly has far-reaching effects upon all tissues.

The effects of mild protein depletion have been stressed because the milder protein deficiency states have not been given sufficient recognition as contributing factors to the vague ill health often accompanying old age. Severe protein deficiencies are also encountered, especially in those undergoing surgery, and they cause the same effects, though probably with graver prognosis, as in younger individuals. However, it is the mild protein deficiencies which are relatively more common and which may contribute to a greater extent than is realized to keeping many elderly people below par for their age.

Because of well-established likes and dislikes, pronounced idiosyncrasies towards certain foods and unwillingness to cooperate, correction of protein deficiency states in the aged by means of diet is often difficult. Patience, tact and firmness are required to impress the patient with the importance of protein intake. In particularly stubborn cases, and in individuals with impaired digestion, protein hydrolysates given as supplements to the diet may be used to provide a considerable proportion of the necessary amount of protein.

Proper nutrition for the aged may mean the difference between infirmity and well-being. Both branches of medical science, nutrition and geriatrics, are developing so rapidly as to permit the hope that healthier and happier old age will soon be realized from mutual application of the advancing knowledge of both.

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Origin of Protein Depletion

Protein under-nutrition in surgical patients arises during one or more of three chronological stages – preoperative, operative, and postoperative.

Any of the factors enumerated previously (see pp. 50 to 63) may bring about body nitrogen depletion preoperatively. The one most commonly seen in all surgical conditions is inadequate protein intake. That is, anorexia and faulty diets are observed irrespective of whether a given patient is a urological, glandular, gynecological, orthopedic or gastrointestinal case. Other factors less commonly seen, though not less important when they do occur, are excessive loss, faulty digestion and absorption, and increased metabolism. Preoperative reduction in plasma proteins due to impairment of plasma protein synthesis is a serious condition and one rather difficult to correct. Fortunately it is one of the functions of the liver for which there appears to be considerable reserve, and it is therefore not so frequently encountered in patients undergoing surgery.

Surgical patients with disorders of the gastrointestinal tract will almost invariably present evidence of either one or all of insufficient intake, faulty digestion or impaired absorption, depending upon the location of the lesion and the correspondingly produced effects and symptoms *(1; 2)*. In patients with stomach ulcers or gall-bladder disease, inadequate intake is usually paramount, whereas in duodenal ulcers, regional ileitis and colitis, insufficient absorption also plays a part. However, the extent of the intestinal absorbing surface is such that only in rare cases will impaired absorption become a serious factor – unless, of course, diarrhea be considered lack of absorption rather than excessive loss.

An item of serious import in one type of gastrointestinal condition, intestinal obstruction, is increased capillary permeability. The acute plasma loss in this connection precipitates shock and hypoproteinemia, which, in addition to the accompanying dehydration, produces a complex blood picture difficult to interpret (see pp. 50-52) *(3)*.

Surgical cases with cancer of the gastrointestinal tract often suffer from reduced intake, because of anorexia as well as impaired digestion and absorption. It has also been shown *(4-6)* that liver insufficiency secondary to carcinoma plays a role in the genesis of the hypoproteinemia seen so commonly in these patients. Another type of patient in whom liver function may be disturbed is the hyperthyroid individual, but here, also, an additional item of significance exists; this time increased combustion of protein consequent upon increased metabolism.

When chronic infection, in conjunction with fever and drainage, continues for some time prior to an operation, nitrogen losses are apt to be large unless counterbalanced by an unusually ample protein intake. Even then, toxic capillary permeability may permit transudation of sufficient amounts of plasma protein for hypoproteinemia to develop suddenly in these infectious cases.

Accidental injuries are followed by a considerable rise in nitrogen excretion, which may be sustained for several days thereafter. Fracture cases, especially, go into negative nitrogen balance following injury *(7)*. If these patients also develop shock, the nitrogen imbalance becomes further complicated, and such patients enter the operating room in a serious negative state with regard to their nitrogen needs.

During operation, protein losses may be considerable. Surgical manipulation itself, even of the most gentle sort, is traumatic. This 'toxic destruction of protein' was stressed several

years ago *(8)* with observation of urinary nitrogen losses of from 10 to 26 grams daily postoperatively despite the administration of 1660 calories of intravenous glucose. More recent confirmation of the general occurrence of this phenomenon postoperatively has appeared *(9 ; 10)*.

Blood loss during operation may be mild or fairly severe without causing extensive protein depletion if protein reserves be adequate. If, however, reserves are reduced by preoperative factors, hemorrhage alone or together with trauma and anesthesia may be significant enough to cause acute hypoproteinemia during operation. Hemorrhagic shock, in the opinion of some investigators, is due, not so much to the loss of red blood cells, as to the loss of blood volume. Plasma loss through hemorrhage, therefore, is a primary manifestation in the etiology of the hemorrhagic shock which ensues *(11)*.

In the development of surgical shock, acute hypoproteinemia has been shown to play a pathologic role *(12)*. Shock, being an acute circulatory impairment, indicates that blood volume is reduced. In minor cases, transudation of blood protein is not excessive, but when the increase in capillary permeability is such that large amounts of plasma are lost, hypoproteinemia becomes an important part of the shock phenomenon.

Anesthesia is a hazard to normal nitrogen metabolism in several ways. Nitrous oxide, chloroform and ether all produce increased tissue protein breakdown, as evidenced by increased urinary nitrogen *(13)*. Another factor secondarily involved in anesthesia is anoxia, which creates plasma loss through alterations in capillary permeability. However, it is the side action of anesthetics on the liver, the organ most vulnerable to attack by these drugs, which is of special concern to surgeons, for, directly related to decreased liver function is a rise in postoperative morbidity and mortality *(14)*. One of the manifesta-

tions of impaired liver function is reduced plasma protein production. A direct relationship between the duration of anesthesia and the incidence of plasma protein decline, despite other variables such as hemorrhage and shock, has recently been demonstrated *(15)*. Fifty percent of the cases under anesthesia for 30 minutes showed a decrease in plasma proteins of more than 0.5 gm.; 70% of those receiving anesthesia for one hour also showed declines, while all patients under anesthesia for 130 minutes or longer became hypoproteinemic. Those patients who showed decreased liver function preoperatively, as determined by the hippuric acid test, invariably had reduced plasma protein levels following anesthesia and operation, whatever the time length.

Changes in blood amino acid levels following nitrous oxide and ether anesthesia have been reported by Farr and his coworkers *(16)*. The average concentration decreased 0.38 mg. per 100 cc. following anesthesia, with a further decline of 0.46 mg. during operation, and a still further reduction in most cases two hours postoperatively. Most of the patients (children) underwent orthopedic surgery. What significance, clinical and otherwise, there may be in such postanesthetic and postoperative hypoaminoacidemia has not yet been determined.

Postoperatively there is a carry-over of the effects of the operation upon nitrogen metabolism. Thus large urinary nitrogen losses, greater than can be accounted for by lack of food calories and protein, appear the first few days. A study of nitrogen balances *(10)* in 41 surgical patients showed that 36 of them sustained net losses of nitrogen ranging from less than 4 gm. to almost 176 gm. ten days postoperatively. Eighteen of them lost an average of 21.31 gm. and eleven lost an average of 89.45 gm., these figures corresponding to 1.5 and 5 pounds of tissue respectively. In the case of a patient who lost 175 gm. of nitrogen following gastric resection, this loss represented

over twelve pounds of tissue. Added to such losses of nitrogen from inadequate intake and operative procedures may be the increased nitrogen loss incurred because of fever, vomiting, hemorrhage and surgical drainage; in apposition to this decreased protein supply, is, of course, the increased need for protein for reparative purposes. Though postoperative protein needs are larger than normal, the intake is usually smaller than normal (further aggravated by impaired utilization), thereby producing a greater discrepancy between the two than if even one were maintained at average levels. Obviously, since the need cannot be altered, intake must be increased.

In viewing the causes of plasma protein depletion in surgical cases, one might classify them as acute or chronic. Under this category, the preoperative causes are usually chronic, the operative, acute and the postoperative, subacute or chronic *(12)*. Acute hypoproteinemia may correct itself if reserves are ample, but chronic hypoproteinemia indicates exhausted reserve stores.

Incidence of Hypoproteinemia

Few statistics are available to show how many patients are hypoproteinemic preoperatively and to what extent surgery further reduces plasma protein levels. A report *(15)* of 215 unselected surgical cases revealed 68.9% frequency of postoperative decline. Persistence of lowered levels was associated with disturbed liver function. Correlation between duration of operation and incidence of postoperative hypoproteinemia was high.

In another series of 278 patients *(15a)*, 22.3% were hypoproteinemic on admission. With 193 patients followed after surgery, an average drop of 5% in plasma protein concentration was observed; degree of decline roughly correlated with initial values. Consequences of hypoproteinemia were impaired wound healing, poorly functioning stomas, edema,

anorexia and irritability. Over 50% of fatalities occurred among patients showing pre- or post-operative hypoproteinemia.

The extent of hypoproteinemia in patients coming to the hospital for thyroidectomy was found to be 63% in one series of 59 cases *(17)*. This same clinical investigation revealed a direct relation between serum protein levels and the severity of hyperthyroidism in that a two-stage operation was required in 73% of the cases having total serum proteins below 6%.

Effects of Hypoproteinemia

The effects of plasma protein depletion in surgical patients are identical with those in the purely medical cases. (See p. 63.) Because certain features predominate in the surgical patient, however, it might be well to emphasize these briefly.

The symptoms of peripheral and subcutaneous edema cause discomfort and irritation to the patient, it is true, but far more important medically is the effect of water-logging upon internal viscera. Pulmonary edema, in the absence of cardiac decompensation, may occur as a result of hypoproteinemia even though the patient be in negative water balance *(18; 19)*. Such edematous involvement of the lungs predisposes to pneumonia and respiratory failure *(20)*. The fatal outcome in a number of hypoproteinemic individuals subjected to operation has been traced to pulmonary failure following edema *(21; 19)*.

Edema of the gastrointestinal tract has been observed to cause diarrhea and vomiting, both of which contribute further to whatever degree of malnutrition may be present *(22)*. Gastric emptying time increases as a result of hypoproteinemia *(23)* and small intestinal motility is retarded *(24; cf. 24a)*. These changes in the normal function of the alimentary canal assume special significance in that intestinal obstruction is liable to result. Casten and Bodenheimer *(19)* have described

a case with symptoms of intestinal obstruction following subtotal gastrectomy, in which laparotomy revealed only edematous, thickened bowel loops and stoma. Ravdin *(23 ; 25)* was the first to point out that edema at the stomal site may mimic in every way (even roentgenographically) a technical defect of the anastomosis, and he suggests that secondary operations in hypoproteinemic patients may be unnecessary if the protein depletion be corrected. Some doubt has recently been cast, however, upon this assumption of a specific cause and effect relationship between hypoproteinemia and postoperative retention. Chauncey and Gray *(26)* have observed patients with gastric retention without plasma protein depletion as well as patients with hypoproteinemia without gastric retention. The only conclusion which may be drawn at the present time is that, in gastrointestinal cases with either hypoproteinemia or obstruction, the presence of one of these should call attention to the possible co-existence and causal relationship of the other.

Wound healing is a major postoperative goal. Hypoproteinemia can cause wound dehiscence as well as retard the healing time of undisrupted wounds. (See p. 67.)

A good example of the way hypoproteinemia predisposes to infection was noted above in the development of pneumonia following pulmonary edema. As far as decreased detoxicating ability is concerned, liver fat content is of most importance. When liver fat is minimal, liver damage from anesthesia is minimal and liver function is maximal. (See p. 130.) Since dietary protein removes liver fat, absence of the lipotropic action of proteins predisposes to fatty livers. These, in turn, mean impaired liver function and increased susceptibility to toxic agents.

It has been established without doubt that protein depletion is of serious consequence in determining the ultimate outcome

of a surgical procedure. When the influence of a nutritive factor is such that it may alter morbidity and mortality statistics associated with surgery, its detection, prevention and treatment become important concerns of the surgeon.

Detection of Hypoproteinemia

The diagnosis of hypoproteinemia is a simple procedure when laboratory determinations reveal an actually low level of plasma proteins in the blood. A normal concentration, however, does not always guarantee the absence of tissue protein loss nor even of plasma protein reduction itself. This is so because other factors besides the concentration of blood proteins in plasma (blood volume, for example) determine plasma protein depletion. (See pp. 50-52.) In patients dehydrated by vomiting, diarrhea, intestinal obstruction, etc., the protein concentration very likely may appear within the normal range, yet these individuals may be extremely depleted of their protein stores. Surgeons must be especially adept then, at detecting the presence of hypoproteinemia even in the face of apparently normal blood levels.

Hematocrit and plasma protein determinations – certainly one is as necessary as the other in surgical cases – will reveal most instances of dehydration. Occasionally, however, as has been noted in gastrointestinal carcinoma or pyloric stenosis due to ulcer, dehydration may coexist with normal hematocrit and protein levels (in the presence of anemia due to reduced erythrocyte count) *(27)*. Under such circumstances, total plasma volume and total circulating protein determinations would be ideal. Without such detailed laboratory studies, however, preoperative assessment of the patient's nutritional status by careful questioning about dietary intake and recent weight changes will give considerable insight into the probabilities of the existence of protein malnutrition.

Since blood protein levels are maintained at the expense of reserve stores, hypoproteinemia always indicates tissue protein depletion. The operative outlook for the individual who has a normal total circulating blood protein maintained by just a slim reserve margin requires special consideration. In such cases and in cases of incipient edema *(27a)*, increased protein needs and losses and reduced intake incident to operative procedure precipitate acute hypoproteinemia and visible edema. Other times, reserves may just be sufficient to maintain blood protein levels despite operation, but attempts to restore fluid and electrolyte balance may flood the body with so much water and salt that edema suddenly appears *(27)*. Careful analysis of the nutritive state of all surgical patients might well be adopted as a general preoperative routine, not only for determining and preventing the occurrence of hypoproteinemia but also thereby cautioning against the consequences of excessive fluid and salt administration.

Prevention of Protein Depletion

To a large degree, the physician has it within his power to prevent protein depletion. Prophylactically ideal would be the administration of high protein diets for several days or weeks preoperatively. In patients with moderate degrees of protein malnutrition, high protein diets have been used with success. However, it is difficult to feed most patients as much as or more than 125 grams to 150 grams of protein daily, and even if it were possible to give somewhat more than this, such amounts might not be sufficient to completely restock the tissue reserves within a short preoperative period of 3 to 4 days.

The oral use of protein hydrolysates as dietary supplements during the preoperative period has made practical the assimilation of much larger amounts of protein than would ordinarily

be possible *(28)*. Protein hydrolysates require no digestive action by the patient; thereby they spare and rest the digestive tract. In patients with impaired digestion, protein hydrolysates become the only means of providing oral nitrogen nutrition. Their preoperative administration is looked upon by foresighted surgeons as one of the most significant therapeutic advances of recent years. (See p. 162.)

Rarely will alimentary feeding, either orally or orojejunally *(29)*, be impossible preoperatively. Occasionally, however, absorption may be so seriously impaired in certain advanced intestinal conditions that parenteral administration becomes the only means of building up the patient. In some cases food cannot be ingested because of such conditions as esophageal stricture, while in other cases complete rest of the gastrointestinal tract is desirable. Protein hydrolysate solutions which can be injected intravenously in place of blood plasma have been prepared and used successfully in many instances. (See p. 170.)

One aspect of the preoperative administration of high protein diets other than for restocking the protein reserves or increasing the quantity of plasma protein has to do with fortifying the liver *(30)*. It has been shown that the greater the liver lipid content, the greater the organ's vulnerability to toxic agents such as anesthetics. It has likewise been shown that dietary protein is lipotropic, that is, it reduces liver lipid concentration. A high fat content, in which case the liver is not maximally protected, may coexist with a high carbohydrate content. Many workers are of the opinion that whatever efficacy carbohydrate intake may have in reducing liver fat is by virtue of its protein-sparing action.

Experimental work with animals *(31)* and with humans *(32)* has definitely led to the conclusion that a high carbohydrate,

high protein diet is about twice as effective as a high carbohydrate diet in reducing liver fat. Since about 29% of patients with chronic gall-bladder disease have abnormally large amounts of liver lipid, Ravdin *(33)* recently undertook dietary experiments for the purpose of discovering to what extent liver fat in these patients could be reduced preoperatively. The experimental diets contained at least 20% protein (supplied largely by casein), 74% carbohydrate, and not over 6% fat. In control patients, chemical analysis of liver tissue obtained at operation showed an average lipid content of 14.2%, while the average fat content in 37 patients prepared 5 days or longer with the above diet was 4.2%. It is convincingly apparent that preoperative fortification of the liver by means of high protein intakes is effectively accomplished. *(Also see 33a.)*

Treatment of Protein Depletion

Even with careful preoperative care, unforeseen or unexpected hypoproteinemia may develop postoperatively. Besides, preliminary feeding will not always prevent – though it may largely compensate or nullify to a great extent – the immediate postoperative urinary nitrogen loss, the so-called 'toxic' destruction of protein. While in itself, a few days' negative nitrogen balance in a well-nourished individual may not be considered alarming, continued nitrogen loss in patients who are unable to eat for some time, after gastrointestinal tract operations, for example, may presage protein depletion. In such cases the administration of nitrogen becomes highly desirable. Since oral ingestion is usually not feasible immediately following most operations, it is primarily during these intervals (recently shortened to less than 12 hours in many cases) that parenteral administration of nitrogen in the form of plasma protein or protein hydrolysates is indicated. For a variety of physiologic reasons (see p. 168), as well as the cost of large

volumes of plasma, interest has been focused upon the use of protein hydrolysates. First tried by Elman and Weiner in 1939 *(34)*, this form of nitrogenous nourishment has since been shown capable of reducing or preventing postoperative nitrogen loss, of maintaining body weight, of supplying nourishment for tissue protein, and of providing materials for plasma protein formation. (See p. 170.) Following the immediate short period of parenteral alimentation, protein hydrolysates, orally or orojejunally, are being relied on to supply the necessary nitrogen during the first postoperative week. Elman *(35)* reports that oral feeding will be tolerated much earlier than is generally believed, particularly if a short period of intravenous feeding immediately follows surgery.

The nitrogen metabolism of surgical patients is of considerable importance in paving the way to a smooth convalescence. By means of several new therapeutic measures – high protein, high carbohydrate diets; predigested protein hydrolysates for oral and intravenous use – the surgeon now can influence and strengthen nitrogen nutrition to a degree where postoperative morbidity and mortality can be appreciably reduced.

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Moon *(1)* states that one of three developments may be expected in severe burn cases – the patient will die from shock in 48 hours; he will subsequently expire from pneumonia, or he will recover. The fatal outcome has been attributed to many causes, but the one which seems to explain most of the general manifestations and consequences of extensive burns is loss of protein, particularly plasma protein *(2)*.

When an individual is burned, skin tissue destruction obviously occurs first. Increased excretion of urinary nitrogen follows this traumatic protein catabolism *(3)*. A recent study of 22 severely burned patients *(4)* revealed that as much as 45 grams of urinary nitrogen were excreted in 24 hours, an amount equivalent to the catabolism of 280 grams of protein a day.

Simultaneously with cellular tissue damage, skin capillaries also are injured by burns. Following such trauma, capillary walls become abnormally permeable to plasma proteins. Until the damage is repaired, chronic loss of plasma from the blood vessels continues. Proof of such plasma loss has been obtained from blood volume and plasma protein concentration studies, as well as from comparative analyses of the protein content of interstitial serous fluid in normal and burned individuals *(5-8)*.

The hypoproteinemia and reduction of blood volume which follow tissue destruction are the factors largely related to subsequent shock, edema and pneumonia. Shock, it will be recalled, is largely a discrepancy between the vascular bed and the fluid contained therein. Regardless of whether burn shock be initiated by direct traumatic action upon capillary endothelium or by the elaboration of a toxin which affects endothelial permeability, its primary features are loss of fluid and

protein, and hemoconcentration. The development of edema is dependent upon fluid and electrolyte supplies in addition to lack of protein, so its appearance is more likely in cases where injudicious fluid administration is carried out. Pneumonia may follow pulmonary congestion and edema arising from the abnormal fluid balance.

The blood picture following a burn is definitely one of hemoconcentration. Within an hour or two, red blood counts may increase to over six million. Plasma proteins, however, appear normal or low. The extent of plasma protein loss from the blood vessels may be more readily appreciated by a comparison of the average value of about 5% to 6% first seen in a burned patient with that of about 10% or more in a case merely showing extreme dehydration. Following simple dilution of the blood, either by the patient's own fluid reserves or by parenteral glucose or saline, plasma protein values descend sometimes to 4%. In a study of 40 patients with severe burns *(8)*, the subsequent rise of the blood proteins to normal levels was observed to be a slow process, requiring weeks in most cases.

Since the fluid imbalance is initiated by altered capillary permeability, any line of treatment to be followed must take cognizance of the state of the endothelium. There is some evidence indicating that transudation of plasma protein continues for about forty hours after a burn *(9)*. During this time, then, even injected plasma may not entirely remain within the blood vessels. Certainly, saline and dextrose solutions will not stay in the vascular bed, there being no osmotic pressure to hold them there. However, repeated small plasma transfusions will help to prevent the development of excessive hemoconcentration during this period. When the endothelium regains its normal degree of impermeability to plasma protein – usually after forty hours – then restoration of plasma pro-

tein and blood volume may be accomplished by several avenues of approach.

Of prime importance in combating the acute plasma protein deficit following restoration of normal capillary permeability is the intravenous administration of plasma. Formulas based upon hematocrit values, plasma protein concentration and body weight have been set up as guides (see p. 167) for determining the amount of plasma (or protein) to be administered, but often these do not adequately indicate the extent of protein losses *(10-13)*. Furthermore, the patient who survives the first 48 hours enters upon a rather protracted period of convalescence in which large amounts of protein are needed, not only for maintenance of normal plasma protein levels, normal osmotic pressure and normal tissue metabolism but also for the necessary tissue growth and repair. When it is realized that 500 cc. of plasma contain but 35 gm. of plasma protein and that some burn cases may lose more than this amount per hour in the very beginning *(10)*, the impracticability of continued administration of such large amounts of plasma as would be required is evident. (See p. 168.) Also, there has even been some recent data presented showing that large amounts of injected plasma may not be utilized by the body for building new protein tissue but will merely remain within the blood stream and be later catabolized to and excreted as urinary nitrogen *(14)*. This, perhaps, would indicate a limit to the utilization of intravenous plasma as a means of protein alimentation.

Clearly, oral protein ingestion is the soundest way of obtaining continued, adequate and effective protein supplies in a convalescent burn patient. The greater the amount of protein ingested, the greater will be the natural synthesis and replacement of plasma protein, the better will be the general nutrition of the patient, and the faster will be the recovery rate. Unfortunately there are limits to the amount of protein which even

normal individuals will ingest. The appetite of an inactive, ill, burned individual usually is not conducive to even moderate protein intakes. Predigested hydrolyzed protein as a dietary supplement in such cases is theoretically ideal and practical as well, as shown by several recent reports of its successful use in burn cases. Beling and Lee *(15)*, starting on the second day, administered oral protein hydrolysate daily to three severely burned men. Plasma proteins began to rise on the third day, and continued upward thereafter, as may be seen in the average curve of two cases (Figure 4). No plasma was given to either of these patients, but the authors believe that its use during

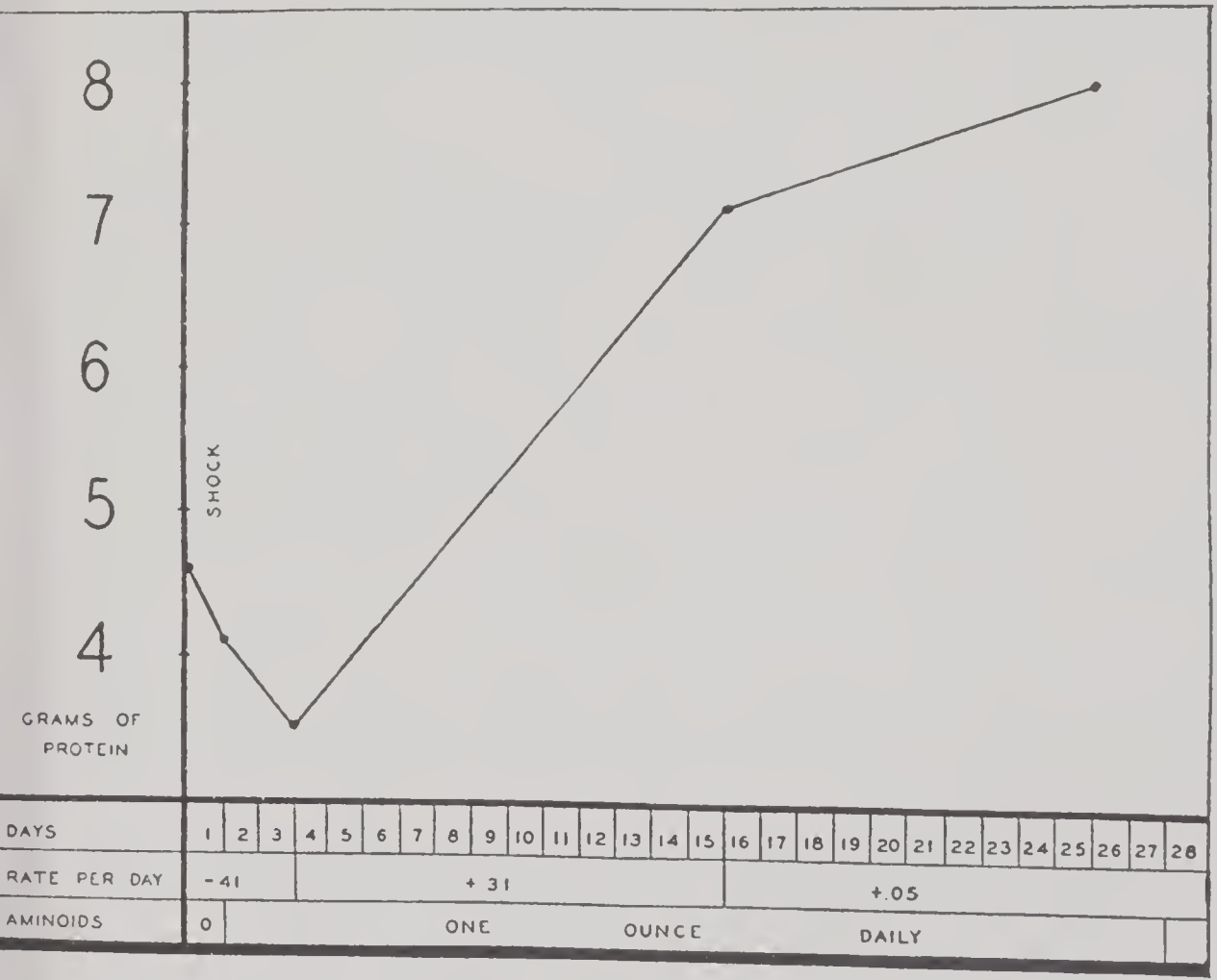

Figure 4. Curve of average plasma protein concentration (grams per 100 cc.) of two burned patients given oral protein hydrolysate therapy *(reproduced from 15)*.

the first few days would have prevented the initial shock and acute hypoproteinemia during that time.

More recently, other investigators *(16; 17)* have published accounts of the successful administration of protein hydrolysate preparations intravenously as well as orally. The effect of protein hydrolysate administration upon the healing of burns was clearly demonstrated in two cases reported by Altshuler and his co-workers *(17)*. The first patient received the preparation orally for 23 days after a six week period of slow healing. Three weeks afterward, the patient was discharged, almost completely healed. In the second case, burns of 7 months duration responded to oral and intravenous protein digest with a markedly accelerated healing rate.

An extension of the effectiveness of protein digests in stimulating the healing of burned surfaces is their administration in cases of poorly healing skin grafts *(15)*. In order for skin grafting to be successful it is apparent that adequate supplies of protein nitrogen are essential for connective tissue proliferation. Skin grafting in patients whose plasma protein level may not have been completely restored to normal may be unsuccessful unless normal protein nitrogen metabolism is established and maintained.

The therapeutic treatment of the systemic effects of a burn in a particular patient is undoubtedly a highly individualized procedure. Each step is conditioned by the blood picture of the patient at the moment. The extent of hemoconcentration must be checked repeatedly by frequent hematocrit readings; simultaneous plasma protein values, interpreted with respect to the hematocrit, will reveal the nitrogenous needs and losses. An excellent clinical picture of the diagnostic and therapeutic measures to be followed in the treatment of severe burns is given in Table VI *(18)*.

TABLE VI—*Course of Patient Aged 27, Weight 55.4 Kg., with 50 per Cent of Body Surface Burned: Calculated Blood Volume 4,261 cc.; Plasma Volume 2,344 cc.*

TIME AFTER BURN	4 HRS.	20 HRS.	25 HRS.	43 HRS.	68 HRS.	5 DAYS	9 DAYS	10 DAYS	12 DAYS	13 DAYS
Blood Pressure	110/60	100/70	100/76	118/96	106/74	120/80	....	....	....	....
Pulse	110	114	103	92	102	90	....	....	....	....
Red Blood Count, millions	....	....	....	....	....	....	3.98	....	3.40	....
Hemoglobin, per cent	89	111	108	103	114	....	64	....	53	....
Hematocrit, per cent	42.8	54.0	53.0	52.3	51.0	44.7	....	....	....	....
Total Plasma Protein, Gm./100 cc.	7.90	7.06	6.70	5.90	6.20	5.75	....	4.80	....	6.30
Albumin, Gm./100 cc.	4.34	4.19	3.90	3.70	3.80	3.32	....	3.00	....	3.30
Globulin, Gm./100 cc.	3.56	2.87	2.80	2.20	2.40	2.43	....	1.80	....	3.00
Albumin-globulin Ratio	1.22	1.46	1.39	1.68	1.60	1.40	....	1.66	....	1.10
Nonprotein Nitrogen, mg./100 cc.	33	43	55	40	60	60	....	31	....	....
Serum Infusion, cc	1,750	1,250	500	250	500	Total 4,250				
Per Cent of Plasma Volume	75	53	22	11	22	Total 183				
Dextrose 5%, Saline 0.9%, cc.	0	0	1,000	4,000	1,000	....	....	....	....	....
Fluids by Mouth, cc	....	150	4,850	3,480	3,810	4,170	....	....	....	....
Total Fluids, cc	1,750	1,400	6,350	7,730	5,310	Total 22,540 cc.				
Urinary Output, cc	....	....	2,820	3.120	1,320	2,280	....	....	....	....
Adrenal Cortex, Extract, 1 cc. intravenously	....	4 times	2 times	1 time						

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Peptic Ulcers

That a correlation may exist between protein deficiency and peptic ulcer was suggested in 1931 by the experimental work of Hoelzel and DaCosta *(1)*. Varying types of dietary and control studies in over 1000 rats led them to conclude that simple protein restriction could produce ulceration of the pro-stomach of these animals. All rats starved intermittently and fed diets low in protein for more than two weeks developed ulcers; inanition or undernutrition in itself was not responsible for the ulcers, for, when an exclusive diet of bran was given, no ulcers were found even though the animals practically starved to death. Similarly, in 1937, Weech and Paige *(2)* observed that the same low protein diet used by them for the purpose of producing hypoproteinemia led to true peptic ulcer in 36% of the rats receiving the diet for an average of 90 days. An additional 23% of the rats showed erosions of the gastric and duodenal epithelium, suggesting, perhaps, incipient ulcers which might have progressed further if the animals had remained on the diet for a longer period of time.

In further substantiation of a relationship between protein nutrition and peptic ulcer is the evidence showing that protein foods will give protection against the experimental production of ulcers. In one reported study *(3)*, four groups of rats were subjected to intermittent periods of starvation and feeding. The control group received a stock diet (a normally adequate diet on which a colony is usually maintained) during the feeding period. Eighty-five percent of the rats in this group developed gastric ulcers. Seventy percent of the rats fed carbohydrates (corn starch or β-lactose, *ad libitum*) during the feeding period also developed ulcers, while 73% of the fat-fed (butter, lard, *ad libitum*) and only 10% of the protein-fed (casein, gelatin,

elastin, or lean meat, *ad libitum*) animals responded with such lesions.

Another rather conclusive experiment *(4)* was performed with Mann-Williamson dogs, animals whose pancreatic and biliary secretions are surgically diverted into the ileum. These dogs, when fed stock diets, usually die from peptic ulcers in an average of 90 days postoperatively. A control group of these animals was given diets in which purified casein supplied 26.6% of the total nitrogen (the rest being furnished by whole and skim milk), while diets differing only in that casein digest was substituted for casein were given to the experimental group. The control animals survived 19 weeks, in which time they lost 47% of their original weight; the hydrolysate-fed dogs lived 32 weeks, losing only 20% of their preoperative weight. These results are highly significant in that this is about the first reported instance wherein the life of a Mann-Williamson dog was materially prolonged by nutritional means. Although the diets did not actually prevent the formation of peptic ulcer, their effectiveness in delaying its appearance suggests that further dietary modifications may yield more satisfactory results.

A rather high incidence of moderate hypoproteinemia in peptic ulcer cases has been reported *(5; 6)*. Whether protein depletion is a causal or resultant factor in peptic ulcer has not yet been clarified. There are certainly logical assumptions for both the 'cause' and the 'result' hypotheses.

The supposed causal, or at least predisposing, effect of protein restriction in the production of gastric and duodenal ulcers may be based upon two gastric phenomenon following protein ingestion; *viz.*: reduction of gastric acidity due to the acid-binding power of proteins and inhibition of peptic activity by increased pH. Thus, simple protein restriction resulted in

higher gastric acidity *(7)*; rats fed diets containing 90% protein did not develop typical ulcerated pro-stomachs even when they were given hydrochloric acid along with the protein *(1)*.

It has also been suggested that alimentary protein may also act by virtue of what has been called "competitive retardation": *i.e.*, the products of a reaction tend to slow up the reaction. Applying this principle to the digestion of proteins, the accumulation of the peptic digestion products would inhibit proteolytic action by pepsin *(3)*, provided such products do accumulate. Since hydrochloric acid and pepsin both have been implicated as causal factors in peptic ulcer, the ability of dietary protein to inhibit the action of both of these may be of some significance.

One other way in which chronic protein restriction may predispose to ulcer formation is in the systemic tissue protein depletion which results. A gastric mucosa with inadequate nitrogen supplies and reserves may be much more vulnerable to ulcer-forming agents.

The manner in which peptic ulcer may bring about protein depletion is readily apparent when one considers the typical restricted ulcer diet. Milk is a good source of protein, but large quantities would have to be ingested in order to supply the entire augmented protein requirement of a sick patient. Furthermore, ambulant peptic ulcer patients, when not under close observation, may restrict their protein as well as vitamin intake to a greater extent than is advisable. In addition, it is not unlikely that digestive, and consequently absorptive, impairment is associated with the gastric lesion of peptic ulcer *(6)*. Although the intake may appear to be adequate in a given case, impaired digestive power may not render the food protein available to the system.

On the assumption of impaired digestion alone, the admin-

istration of predigested protein, that is, protein hydrolysates, to ulcer patients would seem to be a rational way of assuring adequate nitrogen nutrition. There is still another reason, however, which was recently suggested by Levy and Siler *(8)*. They observed that orally administered protein hydrolysate exerted marked buffering action on gastric contents. The pH values of the gastric juice in 18 subjects given 300 cc. of a 10% solution were raised above 4 in all cases, and this effect was maintained for not less than 30 minutes in 13 of the subjects. At a pH of 3.5 or higher, so-called free acidity is no longer present; at a pH between 4 and 5, no more than 10% of the maximum proteolytic activity of pepsin remains. Since values within this range were so readily and easily attained by the protein hydrolysate, its use in the management of peptic ulcer would seem to be an effective method of control.

Dermatologic Ulcers

Ulcers of the skin may arise from a number of causes – bacterial infection, pressure, impaired circulation. Once they develop, they all represent excavations of the integument due to loss of underlying connective tissue. After infectious processes have been halted, the treatment of an ulcer, then, concerns itself basically with stimulation of the growth of fibroblasts. Vitamin C and proteins have both been shown to be essential for fibroblast proliferation (see p. 68); however, vitamin therapy in the absence of adequate protein intake is ineffective *(9)*.

Since proteins stimulate connective tissue growth and reconstruction, ample protein supplies might be effective in preventing as well as treating ulcerous conditions. There is some evidence for this assumption in the case of tropical ulcers; New Guinea coast natives subsisting on high protein diets have a lower incidence of tropical ulcers than inland natives ex-

posed to the same organisms but subsisting on diets low in protein *(10)*.

In a series of 35 cases of decubitus ulcers *(9)*, plasma protein concentrations were found to be subnormal. Restitution of positive nitrogen balance by increasing the protein content of the diets was followed by improvement in the general condition, rise in plasma protein levels and healing of ulcers.

Non-healing ulcers of the varicose, diabetic and decubitus types present a difficult problem in treatment. Patients so afflicted are treated for months and sometimes years with antiseptics, immobilization, heat, etc., all to little avail. Recently Altshuler and his associates *(11)* gave oral protein hydrolysate solutions to ten non-healing ulcer cases. Four were of the varicose ulcer type, three decubitus, two diabetic, and one osteomyelitic in origin. All but one case showed definite evidence of improvement from protein hydrolysate therapy. In the diabetic and varicose cases, particularly, the increase in healing rate was striking. Relatively considered, a therapeutic treatment which produces definite evidence of healing within 3 to 6 weeks in a condition which has remained stationary for a year or longer is promising indeed. The preliminary clinical trials suggest that oral protein hydrolysate therapy, if given in adequate doses and for a sufficiently long period of time, will favorably accelerate the healing of chronic skin ulcers. Successful therapy should include, of course, adequate food and vitamin C intake at all times.

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Section Eleven . . . FOOD ALLERGY

Foods are probably the cause of more allergic phenomena than any other group of allergens. Among the foods, wheat, milk and eggs are the most frequent offenders. It is generally accepted that it is their proteins which provoke such allergic manifestations as asthma, eczema and even arthritis *(1)*.

The hypothesis which explains how food proteins may cause allergic phenomena is based, first, upon the biologic specificity of proteins. Foreign proteins injected into the human blood stream will frequently sensitize the host so that subsequent injection will cause violent anaphylactic reactions. Since the proteins of all species are built up from essentially the same amino acids, it is assumed that species differences lie in the specific organization as well the quantitative make-up of the amino acids in the particular protein molecule. The process of digestion breaks down all proteins to the point where the individual specificity of construction no longer exists. Therefore, the normal individual who would react markedly to the parenteral administration of an animal or other foreign protein would be able to ingest that same protein orally without any such reaction because his digestive processes break that protein down to its constituent amino acids and polypeptides, and it is largely these products of digestion which enter his blood stream rather than the whole protein. In the case of the allergic individual, it is highly possible that an abnormality of the intestinal wall permits the absorption of sufficient undigested or partially digested protein molecules to sensitize the patient so that subsequent entry of the same protein will give rise to allergic symptoms *(1a)*.

Clinical diagnosis often consists in eliminating from the diet suspected or known offenders, while treatment requires omission of these foods from the diet during hyposensitization.

The necessity for careful attention to dietary nitrogen allowance under such conditions is apparent when recognition is accorded the fact that elimination diets are frequently low in protein because allergenic protein foods are excluded *(2)*. Further, elimination diets may have to be adhered to during weeks of diagnosis followed, perhaps, by years of therapy.

The use of protein hydrolysates as a source of nitrogen for allergic individuals was suggested by the work of Shohl and his co-workers *(3)*, who were particularly interested in providing adequate nitrogen intakes for infants and children, in whom milk allergy is a not uncommon occurrence. Protein hydrolysate administered orally, they found, provided positive nitrogen balances similar to those obtained with the usual milk diets. In 1941, Hill *(4)* reported his results in administering a protein hydrolysate product to 36 eczematous infants, all under one year, who were allergic to cow's milk. The feeding mixture which was given for periods as long as three months, contained 20% of protein hydrolysate. In 19 infants, the food was well tolerated, eczema improved, and satisfactory weight gains occurred. Although there is no milk substitute entirely without some disadvantage, the author concluded that the amino acid-containing food mixture is the milk-free food of choice, especially for very young allergic infants.

Beling and Lee *(5)* have reported an interesting case of allergy to cow's milk in an eight month old child. The boy not only presented an erythematous rash, but he also had generalized nutritional edema on admission to the hospital. Plasma proteins were found to be 2.83 gm. per 100 cc. When various diets failed to materially raise this plasma protein level, oral protein hydrolysate was tried. A dose of 2 drachms daily (the diet also contained 15 oz. each of goat's milk and corn syrup, which alone did not maintain plasma protein levels) brought the plasma proteins to 5.08 gm. within 8 days.

By the fifteenth day, complete disappearance of both rash and edema was reported.

The successful use of protein hydrolysates as sources of protein for allergic infants indicates the potential value of such preparations in any individual, young or old, sensitive to a protein food. Particularly will it be of value in those patients who, because of anorexia or dislike for the remaining protein foods they can eat, restrict their protein intake below the safe physiological minimum. However, some individuals are so exquisitely sensitive that reactions may be encountered even with relatively completely digested proteins.

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Section Twelve . . . SHOCK

Although of vital importance as a factor in disturbed fluid, electrolyte and blood protein balance, shock can hardly be counted a clinical entity occurring independently. Rather, shock must be regarded as a virtually inevitable accompaniment of trauma of any origin – accidental, surgical, burn, hemorrhagic, or even psychic. Accordingly the various complications induced by the occurrence of shock have been considered under the headings of the several clinical conditions where shock is most frequently encountered.

Reference has already been made to certain physiological aspects of shock (p. 63). Whether the condition designated as shock is merely the summation of these physiological disturbances or whether there is actual liberation of strong chemical agents (*e.g.*, myohemoglobin) is a problem of etiological significance which, up to the present, has not been settled. The liberation of some toxic chemical agent responsible for at least some, if not all, of the clinical indications of the shock syndrome is a reasonable and attractive working hypothesis. However, experiments made as early as 1927 *(1)* have shown that muscle hemoglobin introduced intravenously in dogs exerts little or no intoxicating effect in doses up to 0.5 to 1 gram per day, and the many recent reports tending to incriminate myohemoglobin as the toxic chemical agent must, then, be viewed in the light of its comparatively low toxicity. In any case, the identity of possible toxic chemical agents would have little therapeutic significance unless means to counter these hypothecated substances became available.

Meanwhile the bulk of experimental evidence, as well as innumerable hospital experiences, indicates that successful therapeutic measures take cognizance of those physiological changes which have been studied. Such measures include initial

plasma transfusions to build up the circulating blood volume with simultaneous increase of the colloid osmotic pressure, whole blood transfusion where excessive hemorrhage might lead to fatal anoxia, and subsequently, more or less intensive protein feeding to combat hypoproteinemia.

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PART FOUR

CORRECTION OF PROTEIN DEPLETION

Brief retrospect of the entire clinical picture presented by protein depletion cannot but lead to the conclusion that, since protein deficiency may arise as a complication of so many other diseases, its occurrence in any patient, whatever his complaint, should never be entirely unsuspected. Inadequate intake, faulty digestion and absorption, excessive loss, increased metabolism, liver impairment – one or more of these may lead either to mild or to severe protein depletion. In such circumstances, blood volume becomes reduced, interstitial fluid volume increases until edema appears, wound healing is retarded, and resistance to infection and intoxication decreases. The effects of protein depletion extend to every living body cell. Correction of protein deficiency states, therefore, is a paramount concern of the physician.

There are several avenues of approach to the treatment of protein depletion. The route of administration and the type of nitrogenous material to be employed both require consideration. In turn, the choice of a method of administration depends upon the extent of the depletion, the relative need, and the situation presented by the patient.

Two broad routes of administration may be distinguished: the enteral and the parenteral; that is, administration into the gastrointestinal tract, or administration by injection into a vein,

subcutaneous tissue, or the sternum. Enteral treatment includes oral administration of proteins or protein digests, while parenteral therapy includes the use of whole blood, serum and plasma transfusions, and protein hydrolysate infusions.

As was stated previously, the choice of method of administration depends upon the type and extent of protein depletion. Chronic and acute deficiencies require different types of treatment, for chronic protein deficiency indicates relatively greater losses of tissue than plasma proteins, while acute protein deficiencies refer to greater losses of circulating protein. Enteral correction is usually indicated in chronic deficiencies, whereas parenteral treatment is needed in acute deficiencies. However, the extent of depletion is also an important determinant of the method of administration. Chronic protein deficiencies may be so severe as to require initial parenteral treatment in addition to enteral correction. In acute plasma protein deficiencies such as are seen in burns, enteral treatment should supplement parenteral transfusions in order to maintain increased protein levels.

The preferred method of treatment of protein deficiencies is the enteral. Normal physiologic processes are duplicated as closely as possible when protein nitrogen enters the body by way of the gastrointestinal tract. Furthermore, parenteral nitrogenous alimentation is not without danger to the patient in that untoward reactions may occur unless the material chosen is non-toxic and non-antigenic, and the injection rate is carefully regulated. However, since enteral alimentation takes longer to restore blood proteins, parenteral administration is indicated for acute deficits in plasma proteins occurring in shock or hemorrhage; its use is limited, though, because of various technical difficulties as well as the danger of thrombus formation.

Since, in the enteral correction of chronic protein depletion, the amount of protein nitrogen to be administered is the same whether one uses the oral, the orojejunal, or the rectal route, the determination of the quantity of material needed will be discussed at this point. One fact of significance in this connection is that in hypoproteinemia due to dietary inadequacy, each gram loss of plasma protein is accompanied by a loss of about 30 grams of tissue protein *(1)*, in accord with the concept of a dynamic equilibrium between the two. When regeneration takes place, only 3.5% of the retained nitrogen is used for replenishment of serum albumin, while 96.5% is utilized for tissue protein replacement *(2)*. In cases of chronic hypoproteinemia, then, enteral treatment is not rapid, and sometimes several weeks intervene before significant increases in plasma protein levels are observed. Unfortunately there is no way of measuring the greater increase in tissue protein which takes place simultaneously.

Elman *(3)* has devised a formula from which the total amount of protein needed can be calculated:

$$\text{Protein need} = \left(\frac{A - SA}{100}\right)\left(\frac{W}{20}\right)(30)\ (K_1) + (K_2)\ (d)$$

where A = Normal serum albumin concentration in gm. per 100 cc., taken as 4.6

SA = actual serum albumin concentration of patient in gm. per 100 cc.

W = patient's body weight in grams

K_1 = the reciprocal of the fraction of ingested nitrogen retained

K_2 = the minimum daily endogenous protein need (usually 25 grams of protein)

d = number of days in which it is proposed to correct the protein depletion

Protein need = total amount of protein in grams which must be ingested during the regeneration period of 'd' days.

The factor, 20, in the denominator converts body weight into plasma volume, while the factor, 30, represents the ratio between plasma and tissue protein loss. Examples of the way in which this formula may be applied are given below:

Example 1: An individual with a weight of 70 kg. and a serum albumin level of 3.1 gm. per 100 cc. is found to retain ½ of the nitrogen ingested. His plasma volume is 3500 cc. (70,000 ÷ 20) and he requires 1.5% [(4.6 — 3.1) ÷ 100] increase in serum albumin. In other words, he needs 52.5 grams of albumin (3500 × 1.5%) to correct his plasma deficit. Since the tissues need 30 times this amount, the total depletion of tissue and plasma proteins in this case is 1575 grams (30 × 52.5). If the patient retains only ½ of ingested protein nitrogen, then K_1 becomes 2, and he must ingest 3150 grams (1575 × 2) of protein in order to replace the 1575 grams depleted from his body. In addition to this amount, the patient also needs enough protein to cover his endogenous needs (25 gm. per day). If it be desired to correct the protein needs of this patient in 3 weeks, then the total amount needed in this period alone for endogenous requirements would be 525 grams (25 gm. daily × 21 days), which, added to the total required to make up the depletion (3150 gm.), reveals a total protein need of 3675 grams. In other words, 175 grams (3675 ÷ 21) of protein would have to be administered daily to correct the patient's protein depletion in 21 days.

Example 2: A patient, weight 56 kg., with serum albumin of 3.5 gm. per 100 cc., retaining 2/3 of the nitrogen ingested, is to be restored to normal level in 10 days.

$$\text{Protein need} = \left(\frac{4.6 - 3.5}{100}\right)\left(\frac{56{,}000}{20}\right)(30)\left(\frac{3}{2}\right) + (25)(10)$$

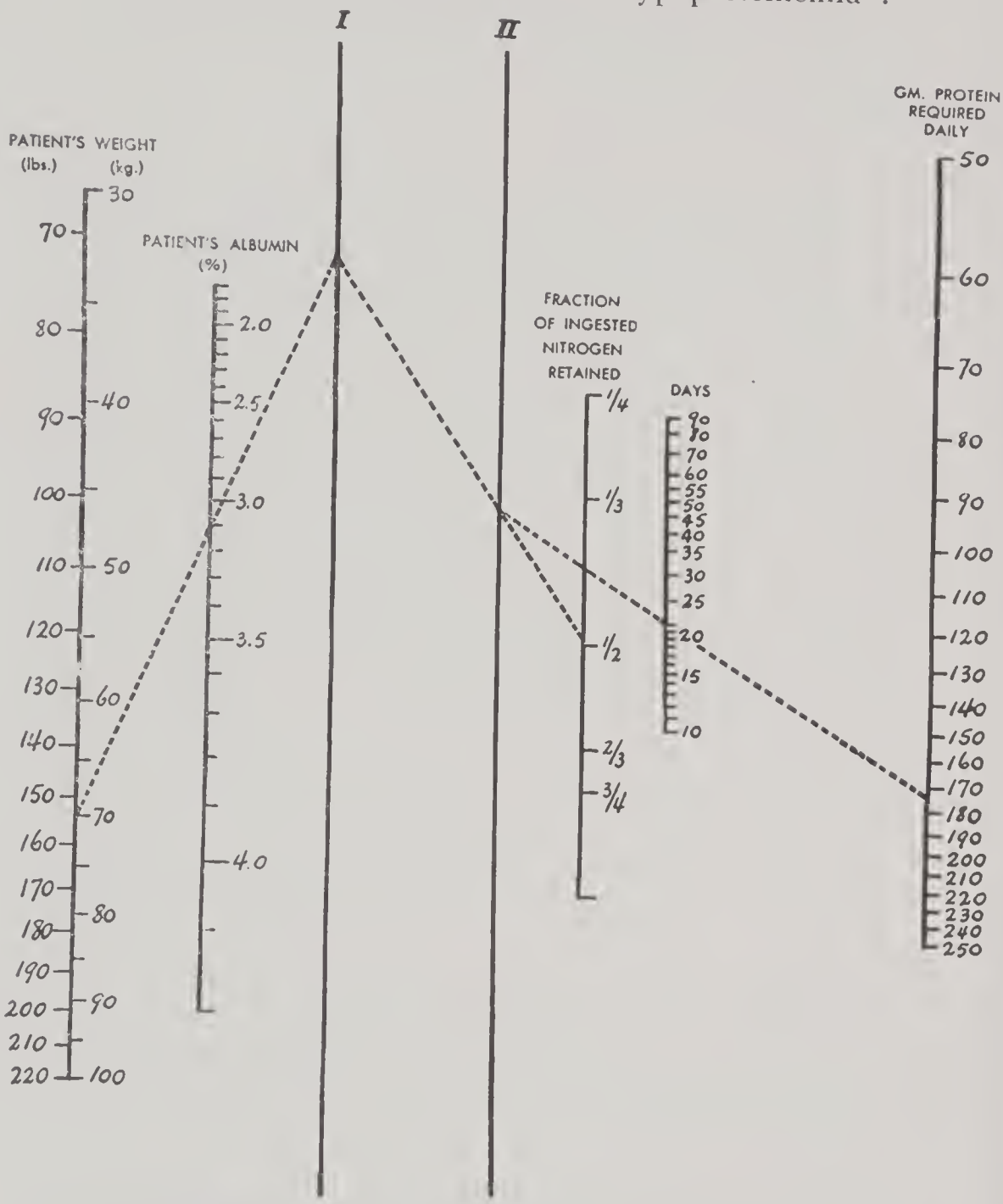
$$= 1636 \text{ gm.}$$

$$\text{Protein need per day} = \frac{1636}{10} = 163.6 \text{ gm. per day.}$$

For convenience, a nomogram based on this formula has been constructed and is reproduced as Figure 5.

It should be emphasized that the above calculations are applicable only to cases of chronic hypoproteinemia brought about by prolonged dietary deficiency of proteins. When hypoproteinemia occurs in connection with hemorrhage, burns, nephrosis, or liver disease, this relationship between tissue and plasma proteins does not hold. Likewise, it is not valid when dehydration is present, for then both the plasma volume calcu-

Figure 5. Nomogram to facilitate computation of daily protein requirement to correct chronic hypoproteinemia*.



*Based on a formula by Elman, R., J. Amer. Diet. Assoc., *18*, 141 (1942).

Example: — A patient weighing 70 kg. has a serum albumin level of 3.1% and retains ½ of the ingested nitrogen. To find the daily protein intake required to correct the hypoalbuminemia in three weeks, connect the patient's weight with the serum albumin concentration and extend the line to guide I. From the point thus located, pass a line through the point corresponding to the fraction of nitrogen retained. A line drawn from the intersection on guide II through the point corresponding to 21 days, and extended to the scale "Gm. Protein Required Daily," gives the answer, 175 gm.

A large scale reproduction of this chart for office use is available from The Arlington Chemical Company on request.

lations based on body weight and the protein concentration determinations will be inaccurate.

For those physicians with insufficient time or facilities for securing the data necessary for using the above formula, the fact that normal individuals require 1 gm. of protein per kg. of body weight for maintenance suggests a basic amount to administer to all patients. According to the clinical signs indicating the extent of malnutrition, an arbitrary decision to administer a 30, 80 or 100 gm. increment can be made. Under these circumstances, of course, it would be difficult to prognosticate the length of time required to correct the depletion.

Once the quantity of protein nitrogen to be administered is calculated or arbitrarily decided, the question as to the means whereby this amount may be administered arises. This involves kind and quality of protein as well as limitations peculiar to the patient.

Oral

Oral ingestion of protein nitrogen is physiologically normal, and usually the most satisfactory method of treating chronic protein depletion. In fact, it is the only route by which maintenance therapy may be continued indefinitely. When oral therapy is to be given, the physician may prescribe high protein diets, protein hydrolysate preparations, or preferably, a combination of both.

All protein depleted patients who can and will ingest proteins should be given a high protein diet. Foods having a high proportion of proteins include meat, milk, eggs, dairy products, fish, soy beans, liver, and wheat (see Table VII). However, the quality of each of these protein foods is not exactly alike since their amino acid composition differs. Two grams of vegetable protein, for example, seem to be necessary to secure

TABLE VII—*Proximate Analyses of Common Foodstuffs*

	PROTEIN	CARBOHYDRATE	FAT
Whole Milk	3.5%	4.9%	3.9%
Skim Milk	3.5	5.0	0.2
American Cheese	23.9	1.7	32.3
Cottage Cheese	19.2	4.3	0.8
Cream Cheese	7.1	1.7	36.9
Eggs	12.8	0.7	11.5
Egg White	10.8	0.8	0
Bacon	12.2	1.4	53.0
Beef Liver	19.7	6.0	3.2
Chicken	21.1	0	4.5
Ham (lean)	19.5	0.3	25.0
Lamb Chops	21.7	0.7	29.9
Pork Chops	14.8	0	32.0
Steak	18.7	1.4	17.0
Veal Chops	18.6	0	15.0
Halibut	18.6	0	5.2
Lobster	16.2	0.5	1.9
Mackerel	18.7	0	12.0
Bread	8.5	52.0	2.0
Dried Lima Beans	20.7	57.3	1.3
Green Lima Beans	7.5	22.0	0.8
Soybean Flour	37.3	9.5	20.2
Green Peas	6.7	15.5	0.4
Corn	3.7	19.7	1.2
White Potatoes	2.0	18.7	0.1
Rice	7.6	79.2	0.3
Dried Brewer's Yeast	46.1	36.6	1.6
Fresh Yeast	13.3	12.7	0.4
Wheat Germ	25.2	47.0	10.0

the same effect in building up plasma protein and reducing edema as one gram of animal protein *(4)*, since the latter supplies a more complete assortment of amino acids. Increasing the quantity of animal protein, therefore, would seem to be a less bulky and more efficient way of augmenting the nitrogen intake. However, the use of increased vegetable protein (wheat, soy beans, etc.) is not without merit when one considers the supplemental effects of 'complete' and 'incomplete' proteins upon each other. (See p. 17.) Furthermore, the present world-wide shortage of animal protein is such that greater amounts of grain protein will have to be relied upon. Whereas formerly 40% to 50% of the dietary protein was furnished by cereal grains, perhaps in the future these will supply 70% to 80% of the protein nitrogen.

A particularly interesting possibility connected with the ingestion of increased quantities of grain proteins involves supplementation; that is, the addition of one protein food rich in a particular amino acid to another protein food deficient in that amino acid. A recent study *(5)* of the supplementation of wheat protein has revealed the tremendous potentialities of this field. For instance, even after enrichment with 8 vitamins and 12 minerals, the growth-promoting ability (for rats) of white flour can be doubled by supplementation with peanut or cottonseed flours, and increased fourfold by supplementation with soy bean flour *(5)*. In consideration of the fact that wheat is one of our most plentiful protein foods, the possibilities of its being supplemented by other grains equally plentiful are particularly promising.

Often it is desirable to have low fat as well as high protein intake. This applies especially to diseases of the biliary tract and liver, and to preoperative fortification of the liver. (See p. 130.) In such situations, skim milk, egg white and cottage cheese satisfactorily fulfill the requirements in that they provide high pro-

tein and low fat intake *(6)*. Similarly, protein hydrolysate preparations contain insignificant amounts of fat.

It is generally accepted that most adult patients will not ingest more than 100 or 150 grams of protein a day. Yet, it has been shown that if they can be made to take more than their usual maximum, either by forced feeding or by means of protein hydrolysate preparations as supplements to their diet, a large part of the increment will be digested, absorbed and utilized. How much protein supplementation may be taken depends upon the relative limits of digestibility and absorbability. Assuming the upper limit of digestibility is reached before that of absorption, protein hydrolysates, preparations containing food protein already digested, could be effectively administered up to the point at which the absorptive limit would be reached. In one recent study *(7)*, infants with acute gastrointestinal disturbances such as vomiting, diarrhea, dehydration and acidosis, were kept in positive nitrogen balance by casein hydrolysate administration, an indication of the absence of absorptive barriers even in the presence of alimentary disorders. A 50% increase in the ordinary nitrogen intake of children (2.4 to 3.6 gm. of protein per kg.) has been easily obtained by adding protein hydrolysate to the diet *(8)*. The absorptive limit, it would seem, is sufficiently high to allow for complete and effective enteral treatment with high protein diets and protein hydrolysate supplements in all cases, except in acute hypoproteinemia where one cannot wait for plasma protein to be synthesized, or in cases having defective liver or impaired absorptive function.

Protein hydrolysates are prepared in the laboratory by means of acid, alkali, or enzyme action. They represent the breakdown of the original protein largely into a mixture of the constituent amino acids and more or less small amounts of polypeptides. Acids destroy tryptophan; alkalies cause racemiza-

tion of amino acids and also destroy cystine and other amino acids depending on conditions of treatment. Preparations derived by the use of either of these agents require supplementation with the missing amino acids. Enzymic hydrolysates do not have this disadvantage, although, on the other hand, hydrolysis is less complete, giving rise to a larger proportion of peptides.

Casein digests have been used to a considerable extent. Casein is a complete protein, and has been shown to be capable of providing positive nitrogen balance, maintaining weight and promoting growth. However, the value of casein is limited by its low content of sulfur amino acids, and when fed as the sole source of dietary nitrogen, it must be given at a level of 18% of the diet in order to assure growth, in contrast to the 9% or 10% level required for lactalbumin and ovalbumin *(9)*. Moreover, since normal diets contain a variety of proteins from several protein foods, a hydrolysate prepared from several protein foods would have a sounder physiological basis than one prepared from a single protein, no matter how 'complete'. "The combination of hydrolysates of various proteins offers great potentialities. It is well known that proteins differ in their amino acid composition, and this makes it possible to select for hydrolysis two proteins which would supplement each other's deficiencies. Thus, one would combine a low cystine, high tryptophan protein with a high cystine, low tryptophan protein, and arrive at an ideal hydrolysate" *(10)*.

The principles of supplementation, therefore, apply equally to protein hydrolysates as to protein foods, and a preparation derived from a single protein would appear to be less nutritionally complete than one prepared from several different proteins. Up to the present time, considerable experimental work has been done with single protein digests and since the results have been promising, it is to be expected that the in-

creased benefits indicated from recent reports on mixed hydrolysates will be further borne out.

Either as the sole source of nitrogen or as a supplement to an inadequate protein diet, protein digests bring about positive nitrogen balance *(11; 8; 12)*. Obviously, if positive nitrogen balance can be obtained, growth will follow *(13)*, and there is even some evidence *(14)* to show that, gram for gram of nitrogen ingested, a protein digest will cause a greater weight increase than the original protein itself. Serum protein regeneration results, also, from administration of protein hydrolysate just as it does from the administration of food protein *(2; 13-15)*. There is no doubt that protein hydrolysates are at least nutritionally equal to the original undigested proteins.

The administration of protein hydrolysates is indicated for those patients in whom whole protein is not properly digested and for those needing amounts of protein larger than can be consumed in the diet. Protein hydrolysate preparations have been used in most of the clinical conditions associated with protein depletion. Some of the conditions which have been reported in the literature so far are nutritional edema *(16)*, allergic states *(17)*, malnutrition *(8; 18)*, liver disease *(19)*, colitis *(8)*, nephrosis *(8)*, chronic active glomerulonephritis *(8)*, hyperthyroidism *(20)*, anorexia *(18)*, cancer of the gastrointestinal tract *(21; 22)*, burns *(16)*, and ulcers *(19)*. Other conditions in which protein hydrolysates may be indicated are anemia, achylia gastrica, hypochlorhydria, achylia pancreatica *(22)*, pregnancy, chronic infections; in short, any protein deficiency state arising primarily as protein deficiency or secondarily from another disease. In surgery, protein hydrolysates are indicated for rapid building up of the nutritional status, for fortifying the liver against anesthesia and operation, and for reducing postoperative morbidity *(23)*.

Supplementary dosage of protein hydrolysate preparations depends upon the protein content of the diet, the particular requirements of the patient, the form of the preparation to be used, and the amount of carbohydrate administered. Simultaneous carbohydrate ingestion is required to release protein nitrogen from the necessity of supplying fuel energy. At least 50% of the total caloric requirement should be in the form of carbohydrate and the carbohydrate-protein ratio should be between 3 and 4 to 1. In a patient whose daily requirement of 100 grams of protein is partly fulfilled by 70 grams of food protein, the dosage of protein hydrolysate would be that amount containing the nitrogen equivalent of 30 grams of protein and the total carbohydrate intake should be between 300 and 400 grams.

In cases of extreme protein deficiency, large amounts of protein digest in conjunction with a high protein diet should be given in frequent feedings, and these, in turn, may both be supplemented further by intravenous nitrogen in the form of plasma or protein digests. The rationale here is analogous to the administration of large doses of vitamins by all possible routes in vitamin deficiency states, the object, of course, being to overcome the deficiency as quickly as possible.

Orojejunal and Jejunal

When passage of food through the mouth, esophagus or stomach is impossible or is not desirable, resort may be had to feeding through an indwelling tube *(24)*. Of course, an ordinary liquid or semi-liquid diet may be given in this manner if the patient's digestive function is unimpaired. In gastrointestinal disorders, however, alimentary rest is often imperative, yet restoration and maintenance of nutrition are equally vital. Both conditions are established when predigested food is introduced directly into the alimentary tract. Thus, preoperative tube

feeding with predigested protein and assimilable carbohydrate strengthens and fortifies debilitated patients unable or unwilling to eat an ordinary diet. Postoperatively, nutrition may be maintained and rapid healing of wounds and anastomoses may be secured without strain upon digestive functions. In resections and anastomoses performed for ulcers, tumors, carcinoma, colitis, etc., tube feeding of predigested nutrients finds its most positive and most common indication *(21; 22)*. It is also valuable in extreme cases of malnutrition accompanied by refusal to eat, as in anorexia nervosa.

Rectal

Rectal alimentation by means of protein hydrolysates and amino acid mixtures has been reported *(25; 26)*. The usefulness of rectal feeding is, however, at best limited because of variabilities in absorption *(27)*.

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Intravenous

Nitrogen can be furnished by whole blood, plasma, serum, or protein hydrolysates. It is important to recognize the difference between the two types of parenteral fluids which these represent. Whole blood, plasma, and serum transfusions supply ready-made plasma proteins, which, upon entry into the blood stream, promptly raise the plasma protein level and exert desirable changes in colloid osmotic pressure. Injected protein hydrolysates, on the other hand, supply amino acids (and peptides) to the blood stream in the same form as food is received from the alimentary tract, the only difference being the site of entry. An increased circulating plasma protein level results only after injected amino acids are synthesized into plasma protein by the liver. Direct replacement of lost plasma protein will be discussed before dealing with the indirect method of replacement by means of protein hydrolysate injections.

As has already been stated, whole blood, serum, and plasma all contain blood proteins. For the correction of hypoproteinemia, whole blood is unnecessary unless anemia coexists; in shock, other than that due to hemorrhage, the use of whole blood only adds to the hemoconcentration. Serum and plasma, therefore, are commonly relied upon for the correction of simple intravascular protein deficits; plasma is used more frequently because of its added antihemorrhagic properties as well as the greater ease with which it may be prepared. In the following discussion, plasma will be referred to as the general prototype of all three preparations.

Plasma transfusions are particularly indicated in acute hypoproteinemic conditions where the deficit in plasma protein is so great as to be critical. In shock due to hemorrhage, burns, surgery, intestinal obstruction, etc., immediate replacement of

lost plasma protein is imperative, for only by restoring intravascular osmotic pressure will fluids be retained within the vascular bed. Dehydration of the blood accompanied by reduction of total plasma protein – in short, loss of plasma – cannot be corrected by fluid administration alone; the osmotic pressure of plasma proteins is required to hold fluid inside the blood vessels.

In those patients with primary idiopathic hypoproteinemia due to impaired fabrication of plasma proteins, the administration of high protein diets or protein digests will not appreciably alter the blood protein level since the difficulty lies in synthesis rather than supply. Until such time as more is known about stimulating or improving the regenerating mechanism, plasma transfusions are the best form of treatment for this relatively rare primary condition. More prevalent by far are cases in which temporary inability to synthesize plasma protein may occur (cirrhosis of the liver, etc.), and in such cases, plasma injections combined with high protein intakes will improve liver function through systemic improvement of fluid balance and tissue nutrition.

With regard to the question of dosage of parenteral fluids in the acute hypoproteinemia of shock, it has been suggested that the protein content be accepted as the standard of dosage since this would eliminate the necessity for stating variations in dilution *(1)*. Thus, 35 gm. of plasma protein rather than 500 cc. of undiluted plasma would be the statement of the usual initial dose in shock. Most formulas by which plasma dosage is computed, however, use volume figures. For example, Harkins *(2)* suggests that 100 cc. of plasma be given for every point of the hematocrit above normal in burn shock. A recently suggested dosage schedule for burns *(3)* is that in which 50 cc. of serum or 60 cc. of plasma are to be given for every percent of body surface burned. During the first 24 hours, an additional

20 cc. to 30 cc. for every percent are given, and another 20 cc. to 30 cc. the first 72 hours. In the 72 hours after a burn, therefore, 90 cc. to 120 cc. of plasma or serum are to be given for each percent of body surface burned.

In massive plasma therapy consideration must be given to the possible toxicity which might be encountered by the introduction of relatively large amounts of citrate ions when sodium citrate is used as the anticoagulant *(4)*. Usual practice calls for the use of 50 cc. of 5% sodium citrate solution per 500 cc. of whole blood. The plasma yield from such a unit may be 250-300 cc. Taking as an average 275 cc., containing, say, 17.5 gm. of plasma proteins, this weight of protein would be accompanied by an average of 0.625 gm. of sodium citrate and a patient receiving 300 gm. of protein would also be infused with more than 10 gm. of sodium citrate.

There are two disadvantages common to whole blood, plasma, and serum transfusions which restrict their use primarily to acute intravascular deficits. The first has to do with the amounts required. For example, 1000 cc. of undiluted plasma contain but 70 gm. of protein. In many cases where plasma proteins are being rapidly lost from the circulation, amounts sufficient to cover the extensive losses cannot be administered because of the time required for intravenous administration of large volumes of plasma. Practically considered, the administration of 150 gm. of protein would require approximately 4000 cc. of whole blood or 2000 cc. of plasma: this amount represents the blood obtained from at least eight donors. Some cases have been reported *(1; cf. 5)* in which an amount of plasma more than three times the normal circulating plasma volume was required to correct hypoalbuminemia.

The second disadvantage to plasma transfusion is that there is some doubt as to the extent to which plasma injections will supply nitrogen for tissue nourishment. Whether the tissue cells accept nitrogen directly in the form of plasma protein or whether plasma protein must first be hydrolyzed down to amino acid aggregates or amino acids themselves is not definitely known. The injection of large amounts of plasma into hypoproteinemic dogs, it has been noted, is followed one week later by increased excretion of nitrogen *(6)*. Other investigators *(7)*, however, using smaller amounts of plasma, report tissue utilization of the plasma nitrogen. Besides the different quantities of

plasma injected, an important variation in experimental technic in these two experiments is the anticoagulant used. In the latter investigations heparinized plasma was injected while the former experiments were done with citrated plasma. *(cf. 4)*. The possibility exists that the large amounts of citrate ion injected in the cases where increased nitrogen excretion was noted might be toxic.

It is possible that the differences observed may also be related to differences in the methods of depletion and in the extent of tissue protein depletion, but these experiments cast some doubt as to the efficiency and directness with which plasma may be used for the correction of tissue protein depletion *(8)*. Plasma, then, is primarily useful in correcting acute and critical protein deficiencies within the blood vessels, but its value as a source of nitrogenous nourishment for the body tissues is impractical and may, in addition, be limited.

Several substances have been suggested as substitutes for plasma. The reinjection of ascitic fluid, which has a high protein content, into patients with cirrhosis of the liver has been accomplished with some success *(9)*, but its general acceptance is limited by the practicalities involved; that is, the strict aseptic precautions necessary for its removal, and the relatively small supplies available. A recently suggested human blood protein derivative, concentrated serum albumin, is presently the object of clinical trial *(10)*. It is doubtful whether such a product will prove practical for regular use, unless the advantages derivable from its employment in modern therapy are much greater than those obtainable with plasma.

Among non-protein plasma substitutes, interest has centered on the use of acacia and pectin. Acacia has been advocated by some clinicians, but the liver damage it produces far outweighs whatever advantages it may possess *(11)*. Pectin solutions have been recently reported *(12)* as effective in the treatment of shock in human subjects, but here, too, the possibility of liver damage may exist. It may be well to emphasize the fact that substances such as acacia and pectin, which exert osmotic pres-

sure and which, therefore, may restore blood volume temporarily at least, in no way correct blood protein depletion. By diluting the blood, they reduce plasma protein concentration *(13)*. Administration of these substances to combat shock, unless supplemented by therapy to increase plasma proteins, does not attack the problem at its base, lack of blood proteins.

Parenteral administration of protein hydrolysate solutions to humans for nitrogen alimentation was first reported by Elman and Weiner in 1939 *(14)*. Successful parenteral use of such hydrolysates in experimental animals has also been recorded *(15-19)*. In humans, protein hydrolysate preparations introduced into the blood stream have been shown to produce positive nitrogen balance *(20-22)* and increased plasma protein *(23; 14)*, and to exert their usual lipotropic effect *(24)*. Using hypoproteinemic dogs (see p. 29), Madden *et al. (24a)* found that a hydrolysate prepared from casein by enzymatic digestion permitted fairly good plasma protein production, while a tryptophan-fortified acid hydrolysate of casein barely provided nitrogen equilibrium and allowed the fabrication of virtually no plasma protein.

Intravenous protein hydrolysates merely substitute for protein nitrogen entering the system from the gastrointestinal tract. The only indications, therefore, for this type of medication are conditions in which enteral feeding is impossible, inadequate or undesirable. In many gastrointestinal conditions, patients may be better prepared for operation by preliminary intravenous feedings. The need for intravenous feeding is most critical in the immediate period following major operations. In operations on the alimentary tract, however, the contraindications to oral feeding may be prolonged. Usually, though, when this is anticipated, an enterostomy tube inserted during operation facilitates and permits postoperative feeding for as long as desired without danger of phlebitis or fear of side-reac-

tions from the intravenous administration of protein hydrolysate solutions.

Besides its uses in surgery, intravenous protein hydrolysate has been used with success in nephrosis and in liver disease. Farr *(25; 26)* has strikingly lowered his mortality statistics of nephrotic crises by the use of protein hydrolysate infusions. Fagin and Zinn *(27)* reported subsidence of peripheral edema, shrinkage in the size of the liver and spleen, and improvement in liver function in five cases of cirrhosis of the liver given 300 cc. of 15% amino acid solution daily for 4 weeks as a supplement to a diet containing 80 grams of protein. A further report *(28)* shows that the injected amino acids possess marked lipotropic activity, the percent of liver fat and protein varying inversely. This further demonstration of lipotropic activity gives added support to the value of preoperative administration of protein hydrolysates, enterally or parenterally, for the purpose of fortifying the liver.

Protein hydrolysate solutions for intravenous use are given simultaneously with glucose solutions in order to spare the amino acids for their more exclusive functions. Five percent solutions with 5% or 10% dextrose are commonly employed. Reactions (flushing, warmth, abdominal pain, nausea) may be largely prevented by careful control of the injection rate. At least ten grams of amino acids can be given in one hour without spilling over into the urine *(29)*. Others *(30)* have reported that the body can assimilate as much as 25 grams per hour. The total daily intravenous dose usually varies from 35 to 130 grams, but some workers have given as much as 300 grams in this manner.

The parenteral administration, to infants, of a mixture of twenty crystalline amino acids has been reported to be as well utilized as a casein hydrolysate *(31)*. Animal experimental

work *(32)* with a mixture of the ten 'essential' amino acids also has revealed good utilization, even when the rate of injection was very high. This work, although preliminary, suggests promising therapeutic advances in the direction of crystalline amino acid therapy.

Subcutaneous and Intrasternal

Other avenues of administration for protein hydrolysate solutions are the subcutaneous *(33)* and the intrasternal *(34)*. These methods – especially the subcutaneous route – may be useful in patients with difficultly accessible veins, or where parenteral administration is to continue for a protracted period.

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A Prognostication

In any field developing as rapidly as is the study of protein metabolism, striking advances may be expected in the near future. The determination of the amino acids essential for nitrogen equilibrium in adult man will possibly be followed by the discovery of specific amino acid deficiency syndromes. Studies along this line indicate, for example, that valine lack results in striking muscular weakness and arginine lack may cause azoospermia. Future therapy may well be concerned then, with the administration of specific amino acids or specific amino acid mixtures for the correction of these syndromes. The analogy to vitamin deficiency states is particularly apparent here, and Rosenberg's inclusion of the 'essential' amino acids among the 'vitagens' is most apt. However, the analogy parts company in that, in specific amino acid deficiencies, the administration of the missing acid must be accompanied by all the 'essential' amino acids in order for nitrogen balance to be obtained.

Continued exploration of the possibilities of these newer therapeutic agents – protein hydrolysates and crystalline amino acids – promises that medical science will be enabled to advance along vastly widened fronts in the successful treatment of many ailments.

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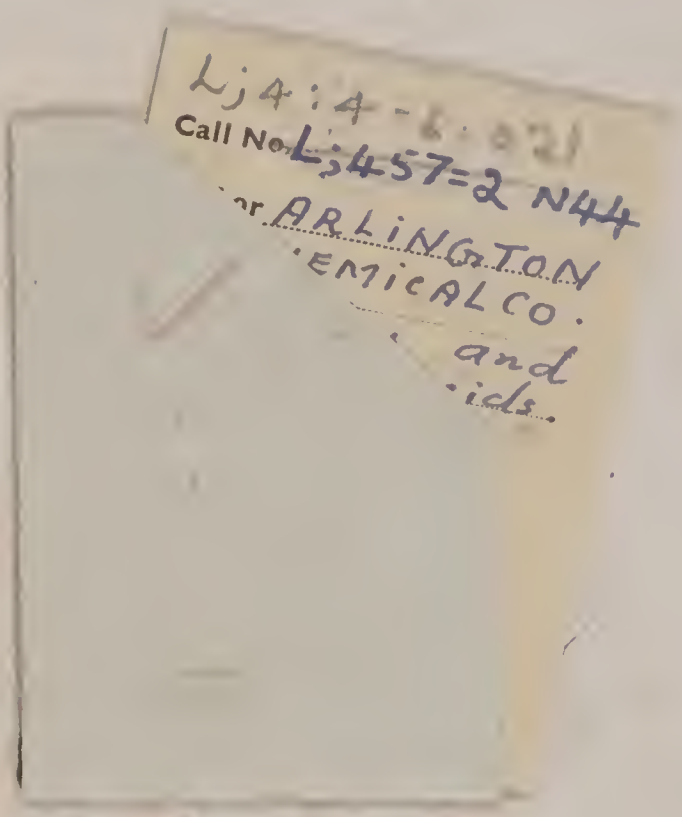
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